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Vector control in a highland province of Burundi: towards a targeted strategy for the prevention of malaria in African highlands

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Vectorcontrole in een hooglandprovincie van Burundi: naar een gerichte strategie voor de preventie van malaria op de Afrikaanse hoogvlakten

Abstract

Highland malaria is a re-emerging problem in Africa since a few decades. Spread of the vectors distribution in time and space exposes the human populations to a longer transmission season resulting in an increased endemicity in the highlands. Besides, deadly epidemics have been reported with higher frequency and amplitude than before. Several factors have been incriminated in this resurgence, including climate and environmental changes, collapsing health systems and vector control measures, and finally an increase in antimalarial drugs resistance. One fifth of the African population lives in malaria epidemic prone areas (desert fringes and highlands) and all age groups are at risk to develop clinical malaria due to their low immune status. The prevention of malaria in these vulnerable populations is one of the priorities for African leaders and international agencies. Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) are the best tools available to reduce malaria morbidity and overall mortality and their impact are even more impressive in low transmission areas such as highlands. In these areas malaria transmission is often patchy where high and low transmission zones alternate. Therefore, several authors have called to focus the vector control interventions only in the zones at high transmission risk, but no evidence is available that such targeted strategies are effective in controlling highland malaria. Questions have also been raised concerning the potential benefit to combine both IRS and ITNs. One additional major concern is the development of insecticide resistance and its impact on the effectiveness of these interventions. The present work considers these different points and tries to provide answers to some of them.

The main objectives of this research were to document the effectiveness and the feasibility of vector control during a malaria outbreak in the highland province of Karuzi (Burundi), to propose a sustainable strategy to control malaria in the highlands, and to evaluate the proposed targeted strategy to reduce malaria in Karuzi. In addition the relative effectiveness of treated nets and spraying when they are combined was investigated. Secondary objectives were to assess the pressure of these interventions in selecting insecticide resistant mosquitoes and to determine the most important risk factors influencing malaria vector densities and malaria prevalence in this highland part of Africa.

Burundi is witnessing the same malaria trend than other highland countries. In **Chapter 2**, the malaria situation in the country is presented since the first malaria record in 1921. After the

end of the “eradication” period that brought the malaria down in most part of the country, an increase of malaria cases have been observed since 1984 ending in a major malaria outbreak in the highland areas in 2000-2001. Malaria is nowadays the main cause of morbidity and mortality in Burundi with 40% of the consultations reported in the health centres and 50% of the hospital deaths in children under five. *Plasmodium falciparum* accounts for more than 90% of all the malaria cases. *Anopheles funestus* and *Anopheles gambiae* are the two principal vectors in the highland provinces, the latter being found in higher density. At high altitudes (>1400m), the relatively low temperature outside, forces the vectors to feed and to rest inside the houses and consequently making them highly vulnerable to vector control activities as IRS and ITN. As it was mentioned, by the end of 2000 the most important malaria epidemic ever recorded hit the Burundian highlands with 2.9 million registered cases over 6 months, for a population of 6.7 million. **Chapter 3** describes the measures implemented to control this epidemic during an emergency situation in the highland province of Karuzi. The high failure rate of the two main antimalarial drugs used for the case management has called for an alternative strategy. IRS and Long lasting insecticidal Nets (LNs) were delivered in the most affected hills. A high coverage especially with spraying was achieved but didn’t curve the epidemic because launched too late. However, the knowledge gained lead to increased preparedness and demonstrated the feasibility of vector control measures in this specific context. Furthermore, this experience was the starting point to design a vector control programme aimed to prevent malaria epidemics in the future. This newly designed vector control strategy, adapted to the specific situation of the Burundian highland province Karuzi, was implemented from 2002 till 2005. IRS and LNs distribution were targeted to the valleys aiming also to protect the population living on the corresponding non treated hill tops. The vector control programme and the nine cross sectional surveys implemented to evaluate its impact on malaria are presented in **chapter 4** and **chapter 5**. The impact on malaria vectors, transmission, malaria prevalence, high parasite density and clinical malaria was assessed and the potential additional effect of nets evaluated. In the intervention valleys, *Anopheles* density and transmission were reduced respectively by 82% and 90% compared to control valleys. In the sprayed areas, *Anopheles* density was further reduced by 79.5% in the houses with nets as compared to houses without them, though this was not translated in an additional impact on transmission or prevalence. The odd of malaria infection was lowered by 45%, high parasite density by 52%, and clinical malaria by 43% in children 1 to 9 years old in the treated valleys when compared to control valleys. Furthermore, history of malaria illness (OR: 0.66) and antimalarial drug use (OR: 0.65) was lower in the intervention compared to control valleys.

The impact of the vector control activities in the older age group was also significant but less pronounced for all these outcomes while the impact was the highest in infants with a reduction of 86% in malaria prevalence. No significant impact, on vector density, malaria transmission or malaria prevalence, was observed in the non treated hill tops of the intervention areas. However, the intervention focused on the high risk areas near the valley floor, where 93% of the vectors were found and 90% of the transmission occurred. Such a targeted strategy could prevent the emergence and spread of an epidemic from these high risk foci.

During the course of this programme the *knock down resistance (kdr*) mutation in *Anopheles gambiae* s.l. was followed from 2002 to 2007. This mutation is often used as a marker of pressure with pyrethroid insecticides. However the link with phenotypic resistance is not well established. Resistance status against pyrethroids insecticide and DDT were also assessed trough insecticide susceptibility tests (bioassays). The results are reported in **Chapter 6**. Before the intervention, the East African *kdr* mutation was present in 1% of the *An. gambiae* s.l. population and by 2007 it reached 85% in the previously treated valleys and 65% in the untreated valleys. It seems that spraying selected for this mutation, but other sources of selection pressure could not be ruled out. In Karuzi, the bioassays showed that *An. funestus* was almost susceptible to all tested insecticides, whereas high reduction in susceptibility was observed for *An. gambiae* s.l. against permethrin and DDT but not against deltamethrin, the main insecticide used during the programme. However, resistance may evolve. Management strategy should be implemented to delay emergence or expansion of insecticide resistance for the sustainability of ongoing malaria prevention programme in Burundi.

Finally, **chapter 7** will give an over view of the relative importance of the malaria risk factors which could help to rationalize interventions in the future. The best predictors for high *Anopheles* density were lower rainfall, absence of vector control measures (spraying and nets), higher minimum temperatures and houses close to breeding sites. *Anopheles* density per houses superior to one, poor housing conditions, and age lower than 39 years old, were associated with higher malaria prevalence.

The present work has shown that controlling malaria epidemic during a complex emergency situation is difficult. However, the implementation of IRS and LN with good coverage is feasible and could be used in prevention of potential epidemics. This six years programme has

allowed to collect useful information on highland malaria and to evaluate the impact of a targeted prevention strategy on malaria. While this strategy has been implemented in one province of the Burundian highlands it can probably be applied with success, in other countries confronted with highland malaria. A sustained vector control effort by the scaling up of IRS or LNs in the malaria high risk areas, improved case management, access to prompt treatment, and a general increase in the living condition of the population, may effectively control malaria in Burundi and in other highland countries.

Samenvatting

Sinds een aantal decennia is hooglandmalaria een opflakkerend probleem in Afrika. Wijzigingen in de temporele en spatiale verspreiding van vectoren stellen de bevolking bloot aan een langer transmissieseizoen met als resultaat een hogere malaria-endemiciteit in deze gebieden. Bovendien worden er de laatste jaren meer en dodelijkere epidemieën waargenomen. Verschillende factoren liggen mogelijk aan de basis van het heropduiken van hooglandmalaria waaronder veranderingen in het klimaat en de omgeving, sociale en economische druk ten gevolge van de bevolkingsaangroei, het in elkaar stuiken van het gezondheidssysteem en van vectorcontrole, en het stijgende probleem van geneesmiddelenresistentie. Eén vijfde van de Afrikaanse bevolking leeft in gebieden waar malaria-epidemieën kunnen voorkomen (grensgebieden van woestijnen en hoogvlakten) waarbij alle leeftijdscategorieën, door hun lage immuniteit, het risico lopen om klinische malaria te ontwikkelen. De preventie van malaria in deze vatbare bevolkingsgroepen is een prioriteit voor Afrikaanse leiders en internationale organisaties. Insecticide geïmpregneerde netten (ITN) en binnenhuisverstuiving (IRS) zijn de best beschikbare methoden om malariamorbiditeit en algemene mortaliteit te verminderen. De impact hiervan is zelfs meer uigesproken in lage transmissie zones zoals hoogvlakten. In deze malaria gebieden is transmissie dikwijls ongelijk verdeeld waarbij hoge en lage transmissiezones elkaar afwisselen. Daarom hebben verschillende auteurs opgeroepen om vectorcontrole interventies enkel uit te voeren in de zones met een hoog transmissierisico. Er is echter geen bewijs beschikbaar dat aantoont dat dergelijke doelgerichte strategieën malaria in de hoogvlakten effectief bestrijden. Bovendien stelt zich de vraag of de combinatie van ITN en IRS een voordeel is in de strijd tegen malaria. De ontwikkeling van insecticidenresistentie en de impact hiervan op de effectiviteit van deze vectorcontrole interventies is eveneens een belangrijke reden tot bezorgdheid. Het onderhavige werk bestudeert deze verschillende punten en tracht hierop een antwoord te geven.

De hoofdobjectieven van dit onderzoek waren het documenteren van de doeltreffendheid en haalbaarheid van vectorcontrole gedurende een uitbraak van malaria in de hoogland provincie Karuzi (Burundi) en het voorstellen van een duurzame strategie voor de controle van malaria in deze hoogvlakten, om de voorgestelde doelgerichte vectorcontrole strategie in Karuzi te evalueren in termen van vermindering in malariatransmissie en -prevalentie. Bovendien werd de impact van de combinatie ‘behandelde muggennetten en binnenhuisverstuiving’ nagegaan,

trends in de frequentie van de *kdr*-mutatie in functie van het verloop van de interventies werden bestudeerd en de belangrijkste variabelen die de densiteit van malariavectoren en malariaprevalentie beïnvloeden werden bepaald.

Burundi is getuige van een gelijkaardige malaria trend zoals andere landen met hoogvlakten. In **hoofdstuk 2** wordt de malariasituatie in het land voorgesteld vanaf de eerste gedocumenteerde malaria in 1921. Na het einde van de 'eradicatie' periode die malaria terugbracht in de meeste delen van het land, werd vanaf 1984 een verhoging van malaria geobserveerd die leidde tot een grote malaria uitbraak in de hoogvlakten in 2000-2001. Malaria is op dit ogenblik de belangrijkste oorzaak van morbiditeit en mortaliteit in Burundi en vertegenwoordigt 40% van de consultaties in gezondheidscentra en 50% van de sterftegevallen in hospitalen bij kinderen onder 5 jaar. *Plasmodium falciparum* is verantwoordelijk voor meer dan 90 % van alle malariagevallen. *Anopheles funestus* en *An. gambiae* zijn de belangrijkste vectoren op de hoogvlakten waarbij *An. gambiae* meer abundant is. Op grote hoogte (> 1400m), waar de buitentemperatuur relatief laag is, worden de vectoren verplicht om zich binnenshuis te voeden en te rusten. Bijgevolg zijn ze zeer vatbaar voor vectorcontrole activiteiten zoals IRS en ITN. Zoals reeds aangehaald kende Burundi in 2000 de ernstigste malaria-epidemie op de hoogvlakten, met 2.9 miljoen malaria gevallen over een periode van zes maanden op een populatie van 6.7 miljoen. **Hoofdstuk 3** beschrijft de maatregelen die werden genomen om de epidemie onder controle te krijgen tijdens een noodsituatie in de hooglandprovincie Karuzi. De hoge falingsgraad van de twee belangrijkste antimalaria geneesmiddelen die gebruikt werden om patiënten te behandelen noopte tot een andere strategie. Binnenhuisverstuiving en *long lasting insecticidal nets* (LNs) werden toegepast op de meest getroffen heuvels. Een hoge dekkingsgraad, voornamelijk van binnenhuisverstuiving, werd bereikt maar had geen effect op de epidemie daar de interventies te laat werden uitgevoerd. De verworven ervaring leidde wel tot een verhoogde paraatheid en toonde dat vectorcontrole mogelijk is in deze specifieke context. Bovendien was deze ervaring het startpunt voor de ontwikkeling van een vectorcontroleprogramma met als doel de preventie van toekomstige malaria-epidemieën. Deze nieuwe vectorcontrolestrategie, aangepast aan de specifieke situatie van de Burundese hoogvlakte provincie Karuzi, werd geïmplementeerd van 2002 tot 2005. IRS en LNs-distributie werden geïmplementeerd in de valleien waarbij het eveneens de bedoeling was om de bevolking die op de nabijgelegen heuveltoppen woont te beschermen. Het vectorcontroleprogramma en de negen *cross sectional surveys* die uitgevoerd werden om de impact op malaria te evalueren worden

beschreven in **hoofdstukken 4 en 5**. De impact op malariavectoren, transmissie en indicatoren, en het potentiële additionele effect van netten werd nagegaan. In de interventievalleien werden de *Anopheles* densiteit en transmissie verminderd met respectievelijk 82 en 90 percent in vergelijking met de controlevalleien. In de IRS-behandelde gebieden werd de *Anopheles* densiteit nog verder verminderd met 79,5% in de huizen met een net ten opzichte van de huizen zonder net. Dit vertaalde zich echter niet in een bijkomende impact op malariatranmissie en –prevalentie. De kans op malaria-infectie werd in de behandelde valleien verminderd met 45%, hoge parasietdensiteit met 52% en klinische malaria met 43% bij kinderen van 1 tot 9 jaar oud t.o.v. de controlevalleien. Bovendien waren de voorbije malaria-aandoeningen (OR: 0.66) en het gebruik van antimalariageneesmiddelen (OR: 0.65) lager in de interventievalleien dan in de controlevalleien. Bij oudere leeftijdsgroepen was voor alle indicatoren de impact van de vectorcontrole-activiteiten eveneens significant, maar minder uitgesproken, terwijl de impact het hoogst was bij zuigelingen met een vermindering van malariaprevalentie met 86%. Er werd geen significante impact op vectordensiteit, malariatransmissie en malariaprevalentie gevonden in de niet behandelde heuveltoppen van de interventiezones. De interventie richtte zich op de risicogebieden in de valleien waar 93% van de vectoren werden gevonden en 90% van de transmissie plaatsvond. Een dergelijke gerichte strategie kon het opduiken en verspreiding van epidemieën vanuit deze risicogebieden voorkomen. Gedurende het verloop van dit programma werd de *kdr* mutatie van 2002 tot 2007 gevolgd. De *knock down resistentie* (kdr) mutatie wordt dikwijls al marker gebruikt voor de druk van pyrethroide insecticiden. Het verband met fenotypische resistentie is echter niet volledig opgehelderd. Het niveau van insecticidenresistentie van pyrethroiden en DDT werd eveneens bepaald met behulp van *bioassay*-testen. De resultaten zijn terug te vinden in **hoofdstuk 6**. Vóór de interventie werd de Oost *kdr*-mutatie bij 1% van de *An. gambiae s.l.* populatie teruggevonden. Ze bereikte 85% in vergelijking met 2007 in de voorheen behandelde valleien en 65% in de onbehandelde valleien. Het blijkt dat binnenhuisverstuiving deze mutatie geselecteerd heeft, maar andere bronnen van selectiedruk zijn niet uit te sluiten. De *bioassays* toonden aan dat *An. funestus* van Karuzi gevoelig was aan alle geteste insecticiden, terwijl een sterke vermindering van de gevoeligheid t.o.v. permethrine en DDT geobserveerd werd bij *An. gambiae s.l.* Er was geen vermindering van de gevoeligheid tegen deltamethrine, het belangrijkste insecticide gebruikt tijdens het programma. Resistentie kan zich verder ontwikkelen. Managementstrategieën zouden uitgevoerd moeten worden om het opduiken of de verspreiding van insecticidenresistentie te vertragen en daardoor de duurzaamheid van de huidige

malariapreventieprogramma's in Burundi te verzekeren. Tot slot geeft **hoofdstuk 7** een overzicht van het relatieve belang van malariarisicofactoren die kunnen bijdragen tot het verder rationaliseren van toekomstige interventies. De beste 'voorspellers' voor hoge *Anopheles* densiteit zijn: minder regen, afwezigheid van vectorcontrole (binnenhuisverstuiving en netten), hogere minimumtemperaturen en huizen dichter bij broedplaatsen. *Anopheles* densiteit per huis hoger dan één, slechte behuizing en leeftijd lager dan 39 jaar werden geassocieerd met hogere malariaprevalentie.

De onderhavige studie heeft aangetoond dat de controle van malaria-epidemieën tijdens een noodsituatie moeilijk is. De implementatie van IRS en LN met een hoge dekkingsgraad is mogelijk en kan gebruikt worden ter preventie van potentiële epidemieën. Dit zes jaar durende programma heeft toegelaten om bruikbare informatie te verzamelen over hooglandmalaria en om de impact van een gerichte strategie ter preventie van malaria te evalueren. Terwijl deze strategie werd geïmplementeerd in één provincie op de Burundese hoogvlakten kan het waarschijnlijk met succes toegepast worden in de andere landen die geconfronteerd worden met hoogvlaktemalaria. Een volgehouden vectorcontrole met behulp van IRS en LN in malaria risicogebieden, snelle en correcte behandeling, en een algemene verbetering van de levensomstandigheden van de bevolking kan malaria effectief controleren in Burundi en in andere landen met hooglandmalaria.

Abbreviations

- ACT Artemisinin based Combination Treatment
- AS Artesunate
- CART Classification And Regression Trees
- CI Confidence Interval
- CQ Chlroroquine
- DDT Dichloro Diphenyl Trichloroethane
- DGCD Direction Générale de la Coopération au Développement
- DR Density Ratio
- ELISA Enzyme Linked Immuno-Sorbent Assay
- EPISTAT Epidemiology and Statistics Cell
- GPS Global Positioning System
- ITM Institute of Tropical Medicine
- ITN Insecticide Treated Nets
- IPT Intermittent Preventive Treatment
- IRD Indoor Resting Density
- IRS Indoor Residual Spraying
- ITS2 Internal Transcribed Spacer 2
- LMTC Projet de Lutte contre les Maladies Transmissibles et Carentielles
- LN Long lasting insecticidal Net
- MoH Ministry of Health
- MSF-B Médecins Sans Frontières – Belgium
- OCHA Office for the Coordination of Humanitarian Affairs
- OR Odds Ratio
- PCR Polymerase Chain Reaction
- RBM Roll Back Malaria
- RDT Rapid Diagnostic Test
- SP Sulphadoxine Pyrimethamine
- SR Sporozoite Rate
- WHO World Health Organization
- WP Wettable Powder

Table of Contents

List of Tables

List of Figures

Chapter 1. General introduction and rational

1.1. The burden of Malaria

Malaria remains one of the most important health problems of our time. There are between 350 and 500 million clinical cases each year [1] resulting in more than 1 million deaths. About 90% of these deaths and 60% of the total cases occurred in Sub-Saharan Africa. In Africa, an estimated 74% of the population lives in areas that are highly endemic for malaria and 19% in epidemic prone areas [2].

Malaria is caused by protozoan parasites of the genus *Plasmodium* and transmitted by *Anopheles* mosquitoes. Four species of malaria parasites affect human health: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*; the first two are the most common. *P. falciparum* malaria is life-threatening especially for individuals with low immunity. The species are not evenly spread across the areas affected by malaria in the world. *P. falciparum* is the most common species and predominates across tropical Africa. *P. vivax* predominates in Asia, South America and the Horn of Africa. *P. ovale* is found in Africa and sporadically in South-east Asia and the western Pacific. *P. malariae* has a similar geographical distribution to *P. falciparum* but with a lower incidence. Human malaria can only be transmitted by the female *Anopheles* mosquitoes. There are around 500 different species of *Anopheles* throughout the world, but only 50 of them are vectors of malaria [3] and 20 species assumed most of the transmission [4]. They tend to bite mainly between sunset and sunrise

1.2. Highland malaria in Africa

1.2.1. Introduction

At the beginning of 1900's, African highlands were considered to have little or no malaria above 1,500 metres [5]. Then changes due to human migration and agricultural development brought effective vectors and parasites into the unaffected areas [6-9]. Epidemics were recorded since 1878 in Madagascar [10], after the First World War in Kenya [11] and Burundi [12], and in 1948 in the Uganda highlands [6]. Due to population growth and increase cultivation of valley floors, malaria became established with high endemicity. Then in the fifties important campaigns for malaria control based on DDT and mass chemoprophylaxis

were implemented in these different highland countries. These measures resulted in reduction or even elimination of malaria in most of these regions. Then, vector control efforts were abandoned until the resurgence of highland malaria in the eighties.

1.2.2. Climate and altitude: determinants for malaria transmission

The transmission of malaria is closely linked to temperature and rainfall. Temperature affects the development, survival and feeding frequency of the *Anopheles*. Furthermore, increased temperatures shorten the parasite development (sporogonic cycle) in the vectors. Optimum conditions for the extrinsic development of the parasite are between 25°C and 30°C (Figure 1). Below 16-19°C, few vectors survive before the completion of the sporogonic cycle and this temperature range is often considered as the threshold for stable malaria. The aquatic stage of anopheline is also temperature dependant. In the laboratory, it was observed, that larval mortality increase considerably when water temperature fell below 18°C [13]. In Kenya highlands only a small larval survival rate was also observed due to the low temperature [14], while the adult mosquitoes could survive inside houses with temperatures 2 to 3 degrees higher [15,16]. Because temperature drops when altitude increases (0.5°C to 0.7°C every 100 meters) [17,18], malaria transmission in the African highland is limited. The upper limit for malaria has been described around 1800-2000 meters in several countries [5]. However, epidemics were recorded exceptionally at higher altitudes [15,19]. Rainfall also plays a crucial role in malaria epidemiology because it provides breeding sites for the aquatic stages of the mosquito's life cycle. In addition, rainfall may increase relative humidity and when it rises above 60%, longevity of the adult mosquitoes increases [18]. By contrast, heavy rains can flush away and kill the larvae [20].

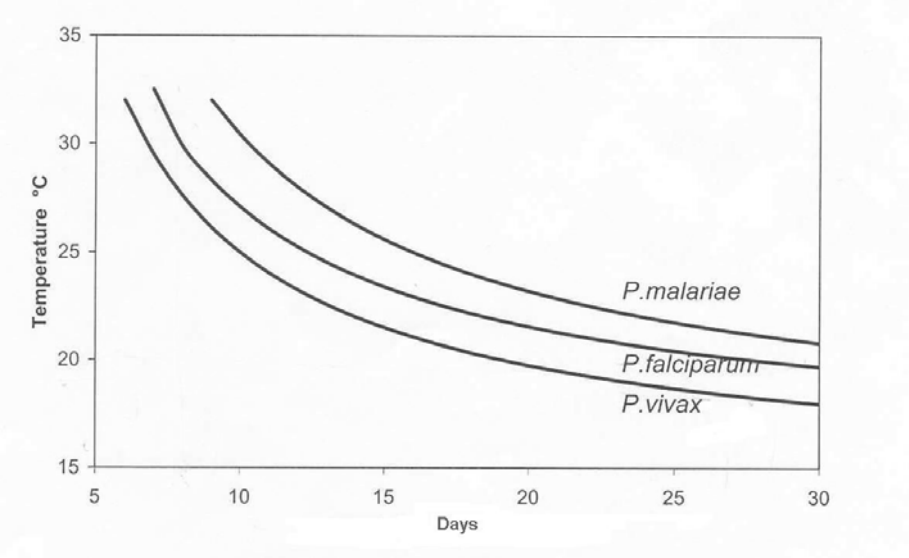

Figure 1: Duration of sporogonic (extrinsic) development of malaria parasites in *Anopheles* according temperature (figure adapted from Warrell et al. [18]).

1.2.3. Highland vectors

The most important vectors recorded in the different African highlands were *Anopheles arabiensis*, *Anopheles gambiae* s.s and *Anopheles funestus* [21-25]. *An. gambiae* s.l. is generally collected in temporary sunlit pools and varies according to seasons, whereas *An. funestus* is breeding in the same ponds or swamps but where vegetation is high and dense. *An. arabiensis* is often found in sympatry with *An.gambiae* s.s. across different countries. However, *An. arabiensis* is usually dominant in dryer places or seasons, while *An. gambiae* s.s. is mostly found in conditions of higher humidity [26,27]. To avoid low temperatures, mosquitoes may find a more favourable climate by resting and feeding indoors. In the highlands, the distribution of the anopheline mosquitoes is more focal than in the lowlands and clustered in the valleys bottoms where the breeding sites are commonly found [28]. Steep hilly slopes [29] and cold nights [17] limit the upward dispersal of adult mosquitoes from the valleys. Understanding the *Anopheles* behaviour in the highlands will help to better target vector control measures.

1.2.4. Epidemiology

Following the spatial and temporal distribution of vectors, malaria transmission and the corresponding malaria infection will be restricted to the warmer months [23] and wet seasons of the year [30,31]. People living nearer the breeding sites (valley floors or dams) are also more at risk (e.g. Kenya [32], Tanzania [17], Ethiopia [33).

P. falciparum is the dominant species found in the highlands [34,35] and is responsible for most of the epidemics [5]. In areas of low or moderate endemicity as in the highlands, the populations have little or no immunity against malaria parasites. Malaria is characterized by high morbidity and mortality among all age groups and such areas are at particular risk for severe epidemics.

1.2.5. Malaria resurgence and epidemics in African highlands

The upper altitude limit for malaria in the African highlands has risen in the past decades and past malaria-free areas have become epidemic-prone [15,19,36]. The spread of the vectors' distribution in time and space exposes the local populations to a longer transmission season and results in an increased endemicity in the highlands [5,8,37]. Different factors can interact to drive these changes, including climate [27], environmental changes such as deforestation [38], irrigation [39] and swamp drainage for cultivation [40,41]. Social and economic pressures as population growth [42,43], collapsing health system [5], antimalarial drugs resistance [44,45] and decrease in vector control activities [46] have also considerably increased the spread of malaria in the highlands. Understanding the impact of these factors on malaria is necessary to improve planning and implementation of national malaria control programmes.

1.3. Malaria prevention and control

1.3.1. Principle

They are two objectives in the management of malaria. First, malaria patients should be diagnosed and treated rapidly with effective drugs. Secondly, people at risk should be protected by one or more prevention measures [47]. We need to distinguished measures aiming a personnal protection and those aiming a community protection. The last one has for objective to reduce malaria transmission and consequently the mortality and morbidity associated to the disease even in unprotected groups. They could be applied, to control epidemics and be implemented punctually or to prevent malaria transmission in the long term. The success of these measures depends on local vector's behaviour, malaria epidemiology, resources availability, and social and cultural practice.

1.3.2. Vector control methods

The main vector control measures are presented in Figure 2 and listed below:

1. **Reducing the breeding sites**
2. **Reducing the larvae population**
3. **Reducing longevity or killing the adult mosquitoes**
4. **Preventing contact between human and vector**

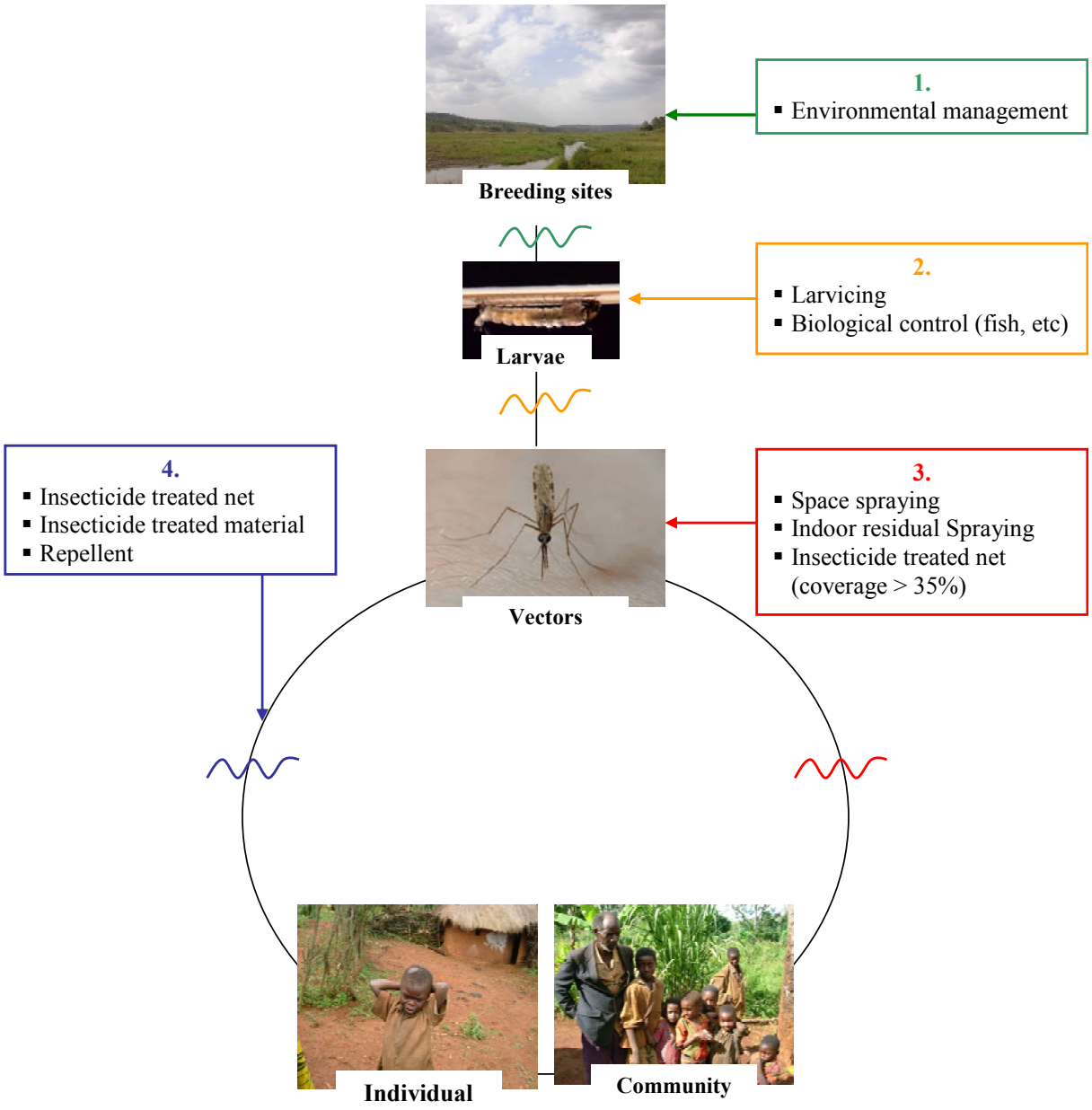

Figure 2: Impact of vector control measures on malaria by targeting environment, vectors, or human (separated in individual and community)

Breeding site reduction and larvicing. These two methods are directed toward the larval stage of *Anopheles*. The objective is to reduce the larvae density and thus the adults through environmental sanitation and water management or chemical and biological control. They have been used before the DDT era with success in different countries [48,49]. However, they require an important knowledge of the vector behaviour and have the best impact in low endemic areas with few and well identified breeding sites [50].

Indoor Residual Spraying. In 1939 with the discovery of DDT, the chemical fight against insects began. Indoor Residual Spraying (IRS) is a method for community protection. It has an effect on reducing longevity and consequently the probability of a vector to become infective but also on vector density by killing mosquitoes resting indoor on the sprayed surfaces. Therefore, the efficacy of IRS will strongly depend on the coverage and on the resting behaviour of the vectors. The success of vector control methods in the temperate countries helped to launch the World Malaria Eradication Campaign (1956-1968) [51]. This campaign achieved elimination in several areas where malaria was unstable (Europe, North America) and house spraying with DDT reduced malaria in most of the tropical countries (central America, Caribbean, Asia, southern part of Africa) but never reached the full eradication [52,53]. The prohibitive cost of the campaigns, combined with the emergence of resistance in the vectors led to the end of the eradication programme. Control replaced eradication and only few countries continue to sustain IRS (Southern Africa and some islands) [54]. However, the re-emergence of malaria in the 1980s has revived interest in these malaria prevention tools with more rational use of new insecticides (carbamates, organophosphates and pyrethroids) [55]. Today, IRS is still an effective tool with high reduction of malaria observed in Equatorial Guinea [56], Madagascar [57], and South Africa [58].

Bed nets were used as a personal protection against bloodsucking insects at night since very early times. Today they remain one of the most important control measures used. Untreated bed-nets can give partial protection against mosquitoes and malaria by reducing man-vector contact [59]. The treatment of the fibre with insecticide has enhanced the protective efficacy of bed nets [60], the insecticide treated net acting like a baiting trap. So far, only pyrethroid insecticides (e.g. permethrin, deltamethrin, lambda-cyhalothrin, alpha-cypemethrin) can be used for impregnation of bed nets because of their fast and high killing effect on mosquitoes and their low mammal toxicity. A review of different trials indicates that ITNs reduced

uncomplicated malaria episodes by 50% in stable areas and by 62% in unstable areas [61]. ITN also has an impact on childhood mortality with a reduction in all-cause of mortality up to 25%. Some authors suspected a delayed or rebound mortality effect but the long-term use of ITN by young children did not shift the mortality to the older ages [62,63]. Scaling up of ITN is a key component of the WHO Roll Back Malaria Initiative but is so far limited to personal protection focusing on the vulnerable groups (children and pregnant women). To benefit from the full potential of ITN, a community effect is required implying a relatively modest coverage of net use (35%-65%) [64]. However, the main constraint to use ITNs on a large scale is the need of impregnation and regular re-impregnation (every 6 months). The development of pre-treated wash-resistant net lasting the life span of the net was a major advance to overcome this problem. Different Long Lasting Insecticidal Nets (LNs) are now on the market and show long term efficacy [65-67].

The cost of ITNs and IRS has been evaluated in different situation. In South Africa [68] and Kenya [69] IRS was cheaper whereas in Tanzania ITN was favoured [70]. Before deciding which methods to use, careful consideration should be given to the relative efficacy, affordability, acceptability and sustainability within the community but also the managerial aspects for the implementation. However, today WHO prone the use of different vector control methods to prevent malaria. IRS and ITNs use have been combined in different settings. Where high coverage is obtained the additional benefit of using both methods is negligible [71] whereas with partial coverage combining IRS and ITN will probably be complementary [72] or even obtain a higher efficacy than one method alone [73]. Using different vector control methods with different classes of insecticide could also be adopted as a resistance management tool and postpone the emergence and spread of resistance.

1.3.3. Insecticide resistance

While for IRS different groups of insecticide can be used, only pyrethroids are appropriate for ITNs. However, some cross-resistance exists between different groups of insecticides and emergence of resistance in vector populations is a major threat for the sustainability of malaria prevention in Africa. Resistance to insecticides in major malaria vectors has become widespread in West [74,75], East [76,77], and Southern African countries [78,79]. Two major mechanisms for resistance exist: the target site resistance, which occurs when the insecticide no longer binds to its target and metabolic based resistance, which is characterized by high

levels or modified activities of three major groups of enzymes (esterases, oxidases or glutathione S-transferase) preventing the insecticide from reaching its site of action [80]. Target site resistance known as knockdown resistance (*kdr*), caused by a single mutation in the sodium channel, is responsible for cross resistance against DDT and pyrethroid insecticides [81,82]. Oxidases and to a lesser extent esterases, are often involved in pyrethroid resistance [83]. The impact of these resistance mechanisms on the vector control efficacy is controversial. In some countries, ITNs still give personal protection against *kdr* resistant *Anopheles* populations [84-86], though, more recent studies showed a reduced efficacy of vector control activities when the *kdr* frequency was high [87-89] or when metabolic resistance mechanisms were probably involved [79].

1.4. Objectives

1.4.1. General objective

Our general objective was to evaluate the impact of a targeted vector control strategy on malaria in the highlands of Burundi and to better understand the highland malaria epidemics and malaria transmission with the analysis of different potential risk factors.

1.4.2. Specific objectives

- To document the effectiveness and the feasibility of vector control during a malaria outbreak and propose a sustainable strategy to control malaria in the highlands
- To evaluate the impact of the targeted prevention programme based on vector conrol measures, on mosquito density, malaria transmission, malaria prevalence, and other malaria indicators in the highlands of Burundi. In addition, to investigate the relative effectivess of IRS and LNs when they are combined.
- To better understand the potential selection pressure and the impact of the *kdr* mutation on resistance and to estimate the resistance of *An. gambiae* s.l. and *An. funestus* against pyrethroids
- To determine the most important risk factors and their relative importance on highland malaria.

1.5. Organisation of the thesis

The present **Chapter** starts with a general introduction on malaria worldwide and focuses on the highland areas. The factors influencing the malaria epidemiology in the highlands during the past decade are presented. The different malaria control methods are reviewed with special attention toward the most widely used: indoor residual spraying and insecticide treated nets. **Chapter 2** reviews the malaria situation in Burundi since the first report at the beginning of the 20th century. Malaria epidemiology and vectors are presented. **Chapter 3** introduces a case study: the interventions done in the highland of Karuzi to control the most important malaria epidemic ever recorded in Burundi. The evaluation of the constraints and opportunities of this intervention is the starting point of the implementation of a four year vector control programme targeting the high malaria risk areas of Karuzi. The evaluation of the impact of this programme on vectors density and malaria transmission is presented in **Chapter 4** and its impact on different malaria indicators in **Chapter 5**. The vector control measures implemented in Karuzi are dependant on insecticide efficacy with emerging resistance potentially compromising the effectiveness of ongoing measures. Therefore, the *knock down* resistance mutation was monitored throughout the programme and the vectors susceptibility to different insecticide was evaluated and reported in **Chapter 6**. In **Chapter 7** an innovative statistical method is used and applied on the Karuzi database to analyse the relative impact of potential risk factors that influence malaria prevalence. A more global picture of malaria is presented and could help to improve malaria surveillance, preparedness and prevention. Finally in **chapter 8**, the most important findings will be summarized and discussed and perspectives for Burundi and other highland countries examined.

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Chapter 2. Malaria situation in Burundi

2.1. Geography, climate and population

Burundi is a small country of 27,834 km² (including the territorial waters of Tanganyika lake, around 2,000 km²) in the great lake region of East central Africa (Figure 3). It is bordered by Rwanda on the North, Tanzania on the South and East, and Republic Democratic of Congo on the West. Burundi lies, between 2°20' and 4°27' South below the equator line and between 28°50' and 30°55' East longitude. The country falls into four distinct geographic regions (Figure 3). The lowlands (700 to 1,000 meters) in the West are called "Imbo" and include the Rusizi River and Lake Tanganyika. The Imbo area is part of the western branch of the Great Rift Valley. Then the mountains of the Congo Nile Crest run north to south and reach an altitude of 2,670 m. Further east is an area of hilly plateaus with lower elevations (1,400 to 2,000 m). In the eastern part of the country, the Kumoso depression (1,200 to 1,400 m) including the Bugesera basin in the North, links the hilly plateaus to the lowland savannahs of Tanzania and Rwanda [1].

The altitude of each region affects the climate, the human settlement, and the agricultural activities. Average annual temperatures vary from 16°C in the mountains and the central plateaus to 24°C in the lowlands near the Lake Tanganyika. Annual rainfall ranges between 900 mm in the lowlands to 2000 mm in the mountains and Burundi has two rainy seasons (February to June and October to December). About 90% of the agriculture is characterized by subsistence crops and 8% by cash crops (coffee, tea and cotton) [1]. In the lowland areas the main cultivated crops are rice, beans, corns, bananas, cassava, oil palm, cotton and Robusta coffee. In the central plateaux corns, beans, sweet potatoes, sorghum, bananas and Arabica coffee are found [1] and since the nineties a variety of rice adapted to high altitude was introduced in the marshy valleys [2]. In the mountains, agriculture is characterized by corns, beans, wheat and tea [1].

The estimated population of Burundi in 2007 is 8.4 million (epidemiological and statistical department of Burundi: EPISTAT). The average population growth rate (2000-2005) is estimated at 3.1%. Approximately 46% of the population is below 15 years old. Traditional settlement is in homestead scattered across the hillside with an average of 300 habitants per

km² which makes it one of the most densely populated countries in Africa. The larger administrative unit in Burundi is the province (17 in the whole country) which is subsequently divided in communes (130 in totals), in hills and in sub-hills, the smallest administrative unit.

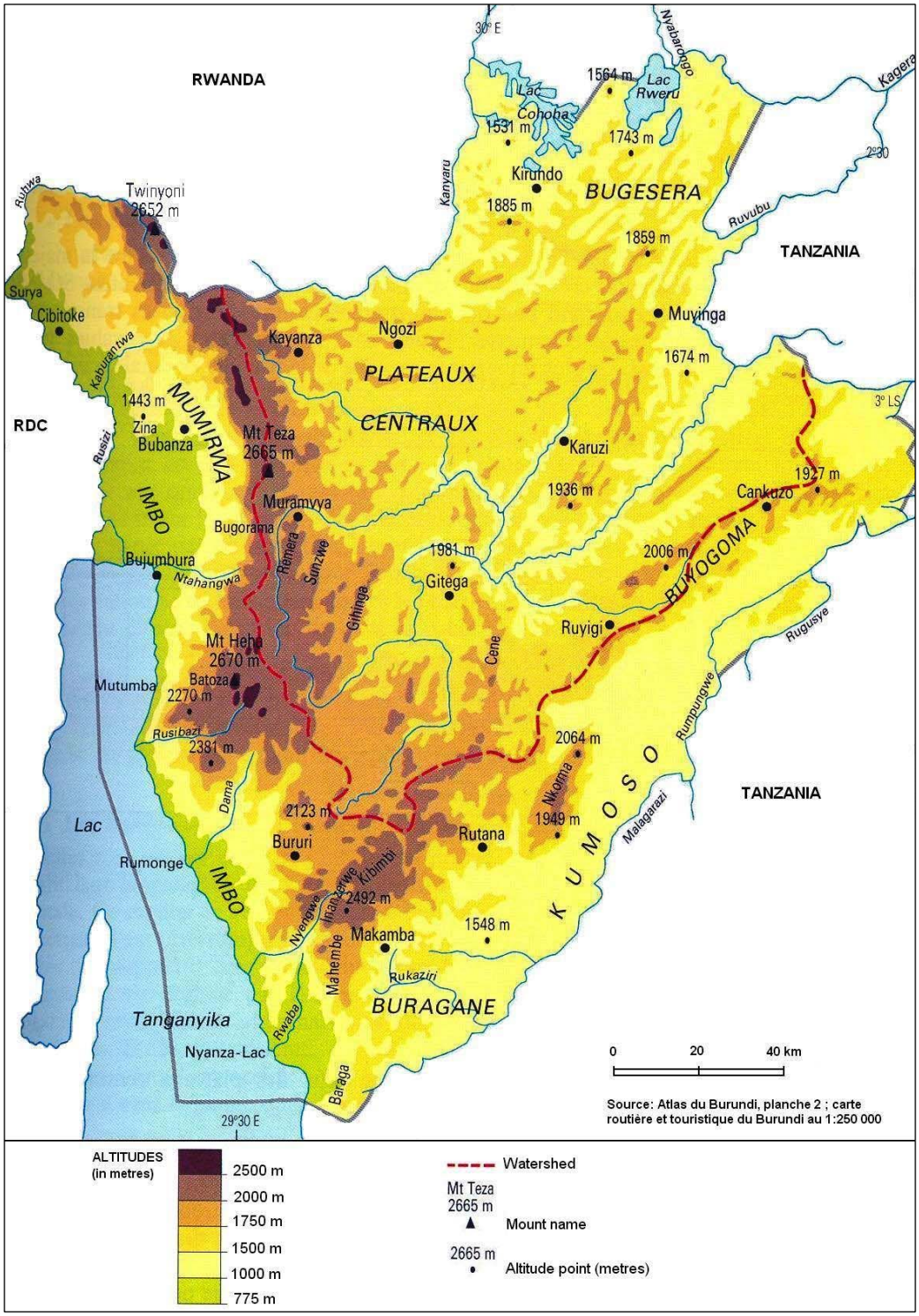

Figure 3: Topographical map of Burundi (Map from the book: Géographie du Burundi, Hatier, France, 1991, ISBN 2-218_3906-0).

2.2. History

Until the XIXe century, the Burundi history comes from an oral tradition and it is admitted that since the XIXe, territory of Burundi had almost the territorial boundary of today. Long before the colonization a king dynasty ruled the country. In 1890, Burundi (along with Rwanda) became part of German East Africa. During World War I, Belgian forces occupied Burundi (1916), and in 1919 it became part of the Belgian League of Nations mandate of Ruanda-Urundi. In 1962 Burundi achieved its independence. Its government was initially a constitutional monarchy, but a military coup brought the military to power. Burundi history has been confronted to multiple conflicts, most notably those beginning in 1972 and 1993, the latter of which lasted 12 years and formally ended with presidential election in 2005. Since, the country has progressed towards reconciliation and reconstruction. However the peace process and the new institutions are still fragile.

2.3. Health system and health situation

The health services had a pyramidal structure; (1) the central level with the Ministry of Health, (2) the intermediate level with 17 health provinces subdivided in 39 health districts, 42 reference hospitals and (3) the peripheral level with 547 health centres in 2004. The ratio is one health centre for 13,000 habitants (WHO norm is 1/10,000) with great disparity between rural and urban areas [3]. Availability of health services is good with 80% of the population living within 5 km from a health facility [4]. In May 2006, the president of Burundi declared free health cares for children below 5 years of age. However, financial constraints limit access for the poorest part of the population and the quality of services is insufficient to address the basic needs. The referral systems are not functioning well due to limited communication and transport from health centres to hospitals. Furthermore many hospitals do not have the staff and the equipment necessary to carry out surgery. Since 2003 onward, a referral system for obstetrical emergencies is progressively set up. According to the Ministry of Health (MoH) 343 doctors (1/20,400, WHO norm 1/10,000) and 2,101 nurses (1/3,300, WHO norm 1/5,000) were working in Burundi in 2003 [3] whereas these numbers are respectively, 200 and 1,348 according WHO [5]. Furthermore, most of the physicians (80%) and 50% of the nurses work in the capital Bujumbura, where only 1/20 of the population is living [3]. The total expenditure on health represents 3.1% of the national budget in 2003, which is far from the 15% proposed in the Abuja declaration.

Malaria is one of the main cause of morbidity and mortality in Burundi with 40% of the consultations reported in the health centres and 50% of the hospital deaths in children under five [4]. In 2007, about 1.9 million malaria cases have been reported by the health facilities. Acute respiratory infection is the second most frequent cause of consultations (10.6%) in health centres for all age groups and represents 9% of the total deaths registered at the hospital [6]. Malnutrition is also an important health problem with 44% of children suffering from chronic malnutrition [4]. Regular outbreak of cholera and meningitis are reported in the lowland plain bordering the Tanganyika Lake. The national HIV/AIDS prevalence in adult was 6% in 2003 [7].

2.4. Malaria epidemiology

Burundi is of particular interest, since on a small spatial scale there is considerable variation in altitude, climatological conditions and land use patterns. As a consequence the epidemiology of malaria varies as well. Data were collected from different sources: local and international papers, reports from the MoH or WHO and data from the epidemiological and statistical department of Burundi (EPISTAT).

2.5. Before 1940

Malaria cases have been observed in the early 20th century, with most of the cases reported from the capital Bujumbura (770 m) [8]. In 1921, a report from the health services of Burundi showed that malaria was observed in the highlands (Gitega 1800 m) where 112 cases over 3174 patients were recorded and a “true” epidemic hit the population at the beginning of the raining season [9]. Malaria has always existed in the lowland but the level of endemicity in the highlands remains unclear. It’s possible that malaria could have been introduced in the highland regions with the soldier displacement during the World War I [10] or it could have existed previously till the altitude of 1700 m with low endemicity [11]. However, it is commonly admitted that since the modification of the hydro-agricultural milieu (1921), accelerated after the famine in 1943, malaria has increased [12,13].

2.5.1. Between 1940 and 1962

In 1948, nine sites from 771 to 2025 meters have been visited and the results of malaria prevalence are reported in Table 1 [14,15]. In the five lowest sites, the malaria was endemic

with usually a relatively low level. The highest site Muramvya (2000-2025 m) had malaria cases probably imported from the surrounding lower areas. In the high plateaus, malaria was mostly confined in the valleys as found in Ngozi, Gitega and Muhinga. The prevalence in the adult population was between 15% and 23% near the rivers and less than 5% for people living higher. In the southeast part of the country, the Kumoso depression (1200 m), prevalence was more than 50%. From 1950, Indoor Residual Spraying (IRS) has been implemented first in the Rusizi plain then in the whole country. In the Rusizi plain, infant (0-1 year) prevalence has been strongly reduced from 49% to 14.6% after the first IRS campaign, to 12.2% after the second and to 2.5% after the third (1957) [16]. The number of recorded cases and deaths were respectively 341,010 and 458 in 1955 and 155,027 and 118 in 1960 in Rwanda-Burundi [17].

Table 1: Malaria prevalence found at different altitude in Burundi.
Data extracted from references [1][15] & [2][14]

		Malaria prevalence	
Site n°	Site name	Children	Adults
1	Bujumbura[1] (771 m)	4 mths-14 yrs 44.2% (42/95)	20% (13/67)
2	Muramvya[1] (2000-2025 m)	6 mths-15 yrs 1.4% (1/72)	6% (3/50)
3	Gitega[1] (1720 m)	6 mths-10 yrs 0% (0/34)	0% (0/50)
4	Gitega: Pont Pecquet[1] (1400-1450 m)	6 wks-15 yrs 30.5% (11/36)	23.3% (9/36)
5	Muhinga[1] (1750 m)	6 wks-15 yrs 10% (9/92)	5% (4/81)
6	Muhinga: Ruvubu river[1] (1400 m)	12 yrs-15 yrs 50% (2/5)	21.1% (8/38)
7	Ngozi[1] (1850 m)	6 mths-10 yrs 8.2% (4/49)	2% (1/50)
8	Ngozi: Bunyongwe river[1] (1500 m)	6 wks-15 yrs 43.2% (41/95)	15.7% (11/70)
9	Kumoso South[2] (1200 m)	1 yr-15 yrs 94.6% (105/111)	51.3% (63/88)

2.5.2. After 1962

After the independence and until 1984, few reports exist on malaria. This coincides with the end of the eradication campaign. Then malaria cases were reported by year until now (Figure

4). The quality of this data are not known and based on clinical symptoms only. However, a constant increase is observed from 1984 (188,230 cases, population of 4,500,000) with the maximum cases recorded in 2001 (3,350,000 cases, population of 6,200,000).

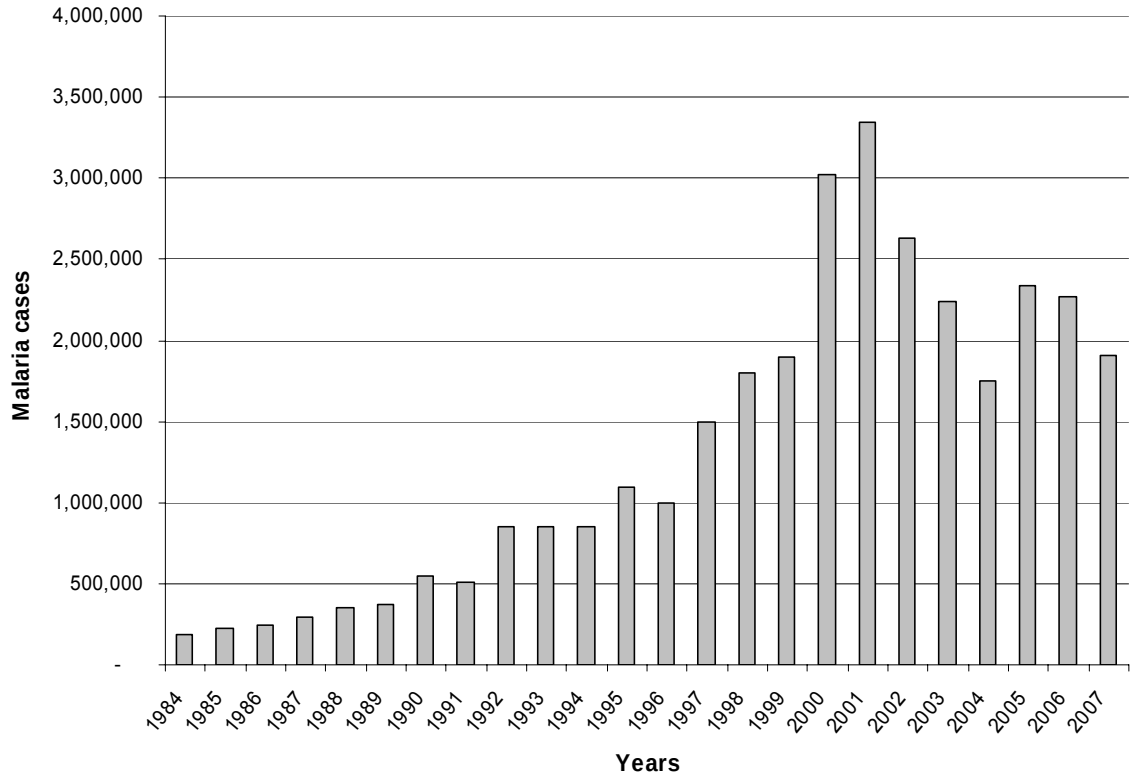

Figure 4: Malaria cases reported by year in Burundi from 1984 to 2007.

In 1989 a stratification of malaria epidemiology was defined using parasitological and spleen indices (Figure 5) [18]. Malaria was found to be endemic in the lowland regions with unstable hyper-endemic area in a small area of central Kumoso and the northern part of Imbo-Nord (prevalence 50 to 75 %) and stable hyper-endemic area in southern part of Imbo-Sud (prevalence 75%). Meso-endemic regions were identified mainly in the North, in the Kumoso, southern part of Imbo-Nord and Imbo-Sud (prevalence 10 to 50%). Malaria was hypo-endemic in the East, in central Imbo and around Bujumbura (prevalence < 10%). The high plateaus and the Congo-Nile Crest were considered non-endemic or epidemic prone. After the increased number of malaria cases, especially in the highlands, the stratification was adapted in 1999 and three main regions were established (Figure 5); 1/Hyperendemic areas where altitude is below 1400 meters, 2/ meso to hypo-endemic areas (altitude between 1400 and 1750 m) and 3/ non-endemic areas with an altitude over 1750 meters (where only imported

cases are reported). The MoH has also identified 8 epidemic provinces: Gitega, Karusi, Kayanza, Muramvya, Muyinga, Mwaro, Ngozi and Cankuzo (Figure 5).

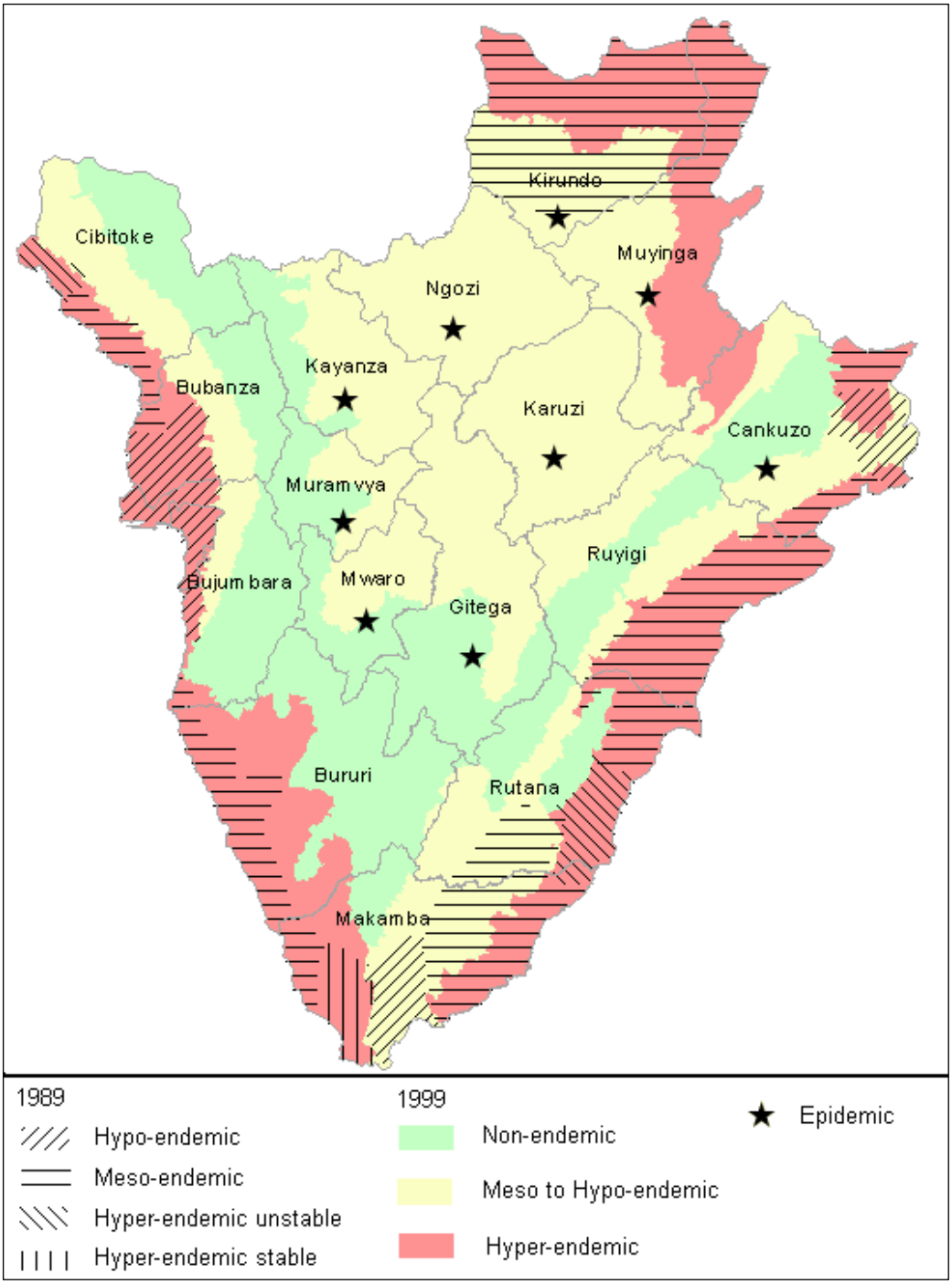

Figure 5: Stratification of malaria areas in Burundi in 1989 and 1999. Localisation of malaria epidemic since 1990 is presented in some highland provinces.

2.5.3. The resurgence of highland malaria and epidemics

Burundi faced, an increase in malaria cases in the whole country since the eighties (Figure 4) and small outbreaks were reported in the highlands in 1991 (Muhanga 1450 m, province Ngozi) [19], in 1992 (Nyabihanga, province Mwaro) and in 1997 (province of Kirundo, 1100-1400 m) [20]. From October 2000 to March 2001, a large malaria epidemic occurred in 7 highland provinces with 2.9 million registered cases over a population of 6.7 million. Between 1,000 and 8,900 probable malaria deaths were reported in the provinces of Karuzi, Kayanza and Ngozi, representing between 51% and 78% of the overall mortality [21]. Karuzi pre-epidemic data from the preceding four years shows a progressive increase of malaria (Figure 6).

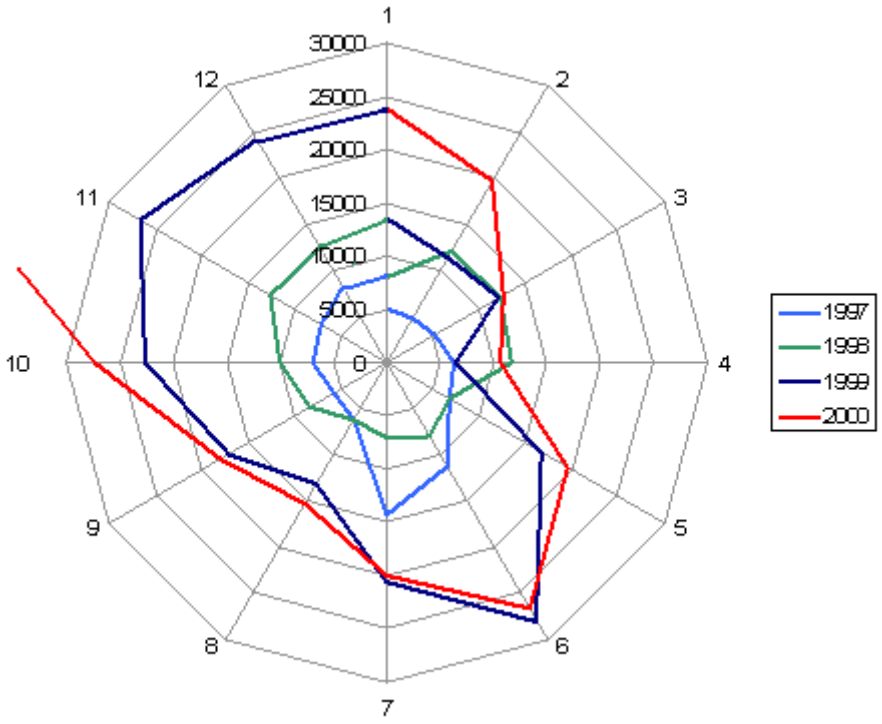

Figure 6: Spiral representation of malaria cases per month in the province of Karuzi (Burundi) from 1997 to 2000.

Temperatures during the six months preceding the outbreak showed no obvious change in average, compared to the last 10 years (Figure 7). An unusual high precipitation occurred at the time of the malaria peak (November) and could not be responsible for triggering the epidemic, but was probably responsible for stopping or decreasing malaria transmission. Breeding sites were probably washed out by this unusually heavy rainfall and the sporogonic cycle increased due to temperatures dropped below 18.5°C as from the end of December onwards, a progressive decrease in malaria was observed. This case seems to follow the thesis

of several authors which argue that climate change is not responsible for the East African highland malaria resurgence [22-24]. The only meteorological factor that indirectly favoured a malaria epidemic is probably the long dry season that increase acute malnutrition especially in young children. This situation must have weakened the immune capacity of the children under five and could explain the unprecedented level of malaria morbidity and mortality [25,26]. Other non-climatic factors such as ecological modifications and population movements could have contributed to the recent changes in malarial epidemiology as in other African highlands [27]. In Burundi, the clearing of forests and swamps for cultivation of irrigated crops has been going on since 1921 [16] and was accelerated in the eighties. Furthermore, due to ten years of civil war, basic health services were in decline and supply of drugs was irregular. This has been compounded by an increasing drug resistance. Several studies conducted in Burundi reported a *P. falciparum* resistance to chloroquine [28,29]. According to Di Perri *et al.* [30] the response of *P. falciparum* to chloroquine decreased from 72.9% in 1992-1993 to 56% in 1994-1995 in children under five. Another study, conducted in 2001 by MSF in the province of Karuzi, showed a treatment failure (day 14) of 93% to chloroquine (CQ) and 66% to sulfadoxine pyrimethamine (SP) suggesting high resistance rates (MSF internal report). The low efficacy of SP and CQ caused a delayed response to antimalarial treatments, a higher rate of recrudescence leading to a larger human reservoir in parasites and gametocytes which accelerated further the spread of resistant parasites [31,32]. The unprecedented magnitude of the epidemic of 2000 could be closely related to the decreasing efficacy of the two main drugs used against malaria [33].

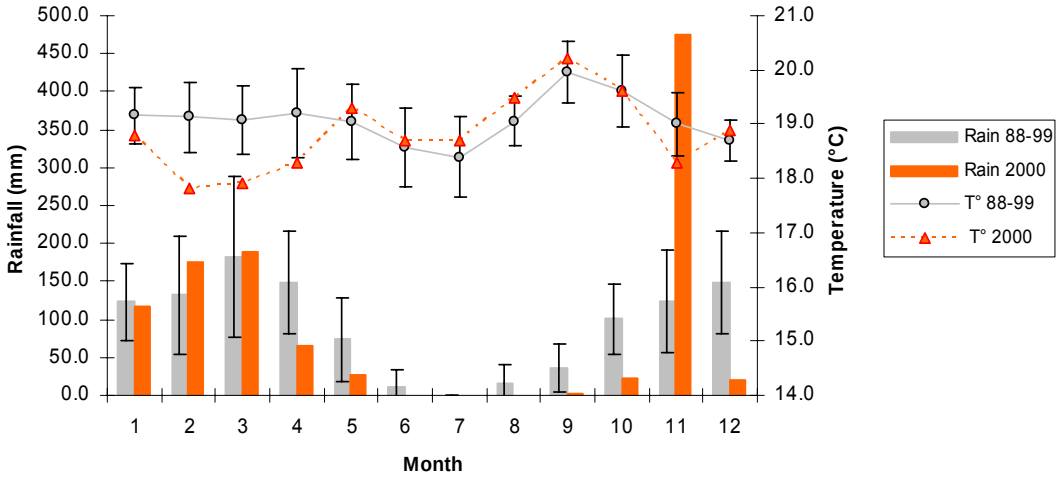

Figure 7: Monthly rainfall and temperature average from the meteorological station in the province of Karuzi (Burundi) from 1988 to 2000.

2.6. Malaria vectors

The first study reporting vector species in Burundi was carried out by Vincke [13] in 1943. In Bujumbura (700 m), less than half of the mosquitoes collected (n=2,824) were *Anopheles* (n=1,287) of which 6.1% were *Anopheles gambiae* s.l. and 47.3% *Anopheles funestus*. In Gitega (1740 m), 2890 *Anopheles* larvae were collected, 16.7% were identified as A*n. gambiae* and 9.9% as *An. funestus*. However, only 42 mosquitoes were collected in houses of whom 50% *An. gambiae* and 2% *An. funestus*. This difference between larval and adult density has been explained in the Kenya highlands by a low larval survival rate due to a development retarded by low temperature [34].

The most important works done on malaria vectors and *Anopheles* in Burundi are presented in Figure 8 summarizing the findings of two papers [10,15]. The principal vectors, *An. gambiae* s.l., *An. funestus*, *An. nili* and *An. moucheti* were found below 1800 meters. The last two species were not reported in other studies.

In 1950, in the Congo part of the Rusizi plain, an unexpected high density of anophelines was found inside the houses (more than 1000 per room) [35] whereby 98 % of the children were found positive for malaria [36]. *An. funestus* was by far the dominant *Anopheles* species (96%), *An. gambiae* s.l. and *An. pharoensis* represented respectively only 3.3% and 0.7% [35]. In the delta of the Rusizi (Burundi side), during a later study in 1981, *An. pharoensis,* a poor malaria vector, was the dominant and most abundant species found (more than 100 bites man night). Densities of *An. gambiae* s.l. and *An. funestus* were very low in the delta inducing a malaria prevalence of less than 5% [37]. *An. ziemanni* and to a lesser extent *An. implexus* are common in the Rusizi valley but are very zoophilic and not incriminated in malaria transmission [37].

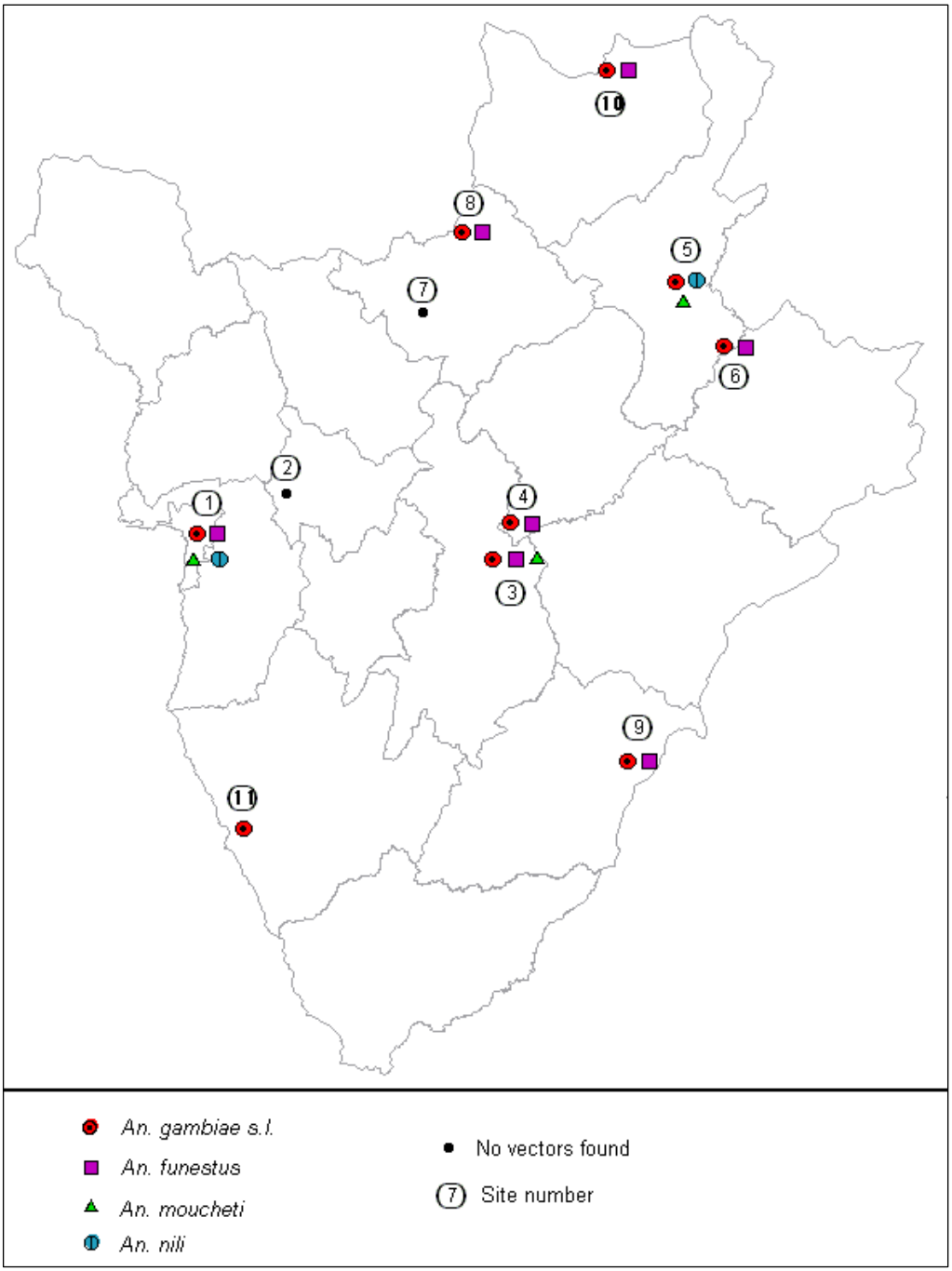

Figure 8: Repartition of malaria vectors in Burundi in 1946 (sites 1-8) [15] and in 1950 (sites 9-11) [10]

In south Imbo and in Karuzi highlands *An. gambiae* s.l. was found to be the dominant vector species while *An. funestus* represented respectively 23% [38] and 17% [39] of the collected *Anopheles*. *An. gambiae* is a complex of six morphologically similar species having different behaviours and consequently different abilities to transmit malaria parasites [40,41]. Of these species only the two most effective vectors were found in Burundi: *An. gambiae* sensu stricto

and *An. arabiensis*. According Lindsay *et al* [42], *An. gambiae* s.s. survives longer in the humid environment than *An. arabiensis,* which fits with the observations made in Burundi. The predominant species in the dryer areas (rainfall 700 mm/year) of the North and central Imbo (Rusizi plain) is *An. arabiensis* (more than 95% of *An. gambiae* s.l. collected) [43-45] whereas in the more humid south Imbo (1000 mm/year) *An. gambiae* s.s. is the only species of the complex found [46]. In the highland province of Karuzi (1400-1900 m and rainfall of 1180 mm/year), 98.2% of the *An. gambiae* complex were *An. gambiae* s.s. [39].

2.7. Malaria control activities and surveillance

From 1920 to 1950 the control activities were based only on passive detection of cases and treatments. From 1952 to 1957, seven cycles of spraying with DDT started in the lowland near the Tanganyika Lake and the urban areas and were extended to the whole country in 1956. At this time, 1 million of houses in Rwanda and Burundi were treated below 2000 meters [47]. Chemoprophylaxis with pyrimethamine (Daraprim®) was given only in 1958 in the Mpanda region (province Bubanza) where malaria prevalence had increased.

The sixties signed the end of the eradication era. Between 1966 and 1984, malaria control activities were ran by the "Mission d'Assainissement de la Plaine de la Rusizi" (MAPR). Teams were still in charge to spray some houses in the Rusizi plain and Kumoso region [47,48], and chemoprophylaxis was distributed in schools until 1982. However these activities were scarced and not coordinated properly.

In 1984, with the help of the Belgium cooperation, a project to control infectious diseases and malnutrition (Lutte contre les Maladies Transmissibles et Carentielles: LMTC) was implemented and targeted the following diseases, malaria, schistosomiasis, onchocerciasis and malnutrition. In 1985, IRS was reintroduced in the Imbo plain. With only one round of IRS per year, before the peak of transmission, malaria parasite prevalence was reduced from 60% in 1985 before the campaign to 5% in 1990 [43]. Distribution of Insecticide Treated Nets (ITNs) was also implemented in the lowland areas of Nyanza Lac (Imbo south) with success. In children under 5, high parasitemiae (<2000 trophozoites /µl) were reduced by 42% and 53% for a coverage of respectively, 55% and 44% [38]. All this activities were stopped due to political unrest.

In 2000, IRS with pyrethroid insecticide was used again in some provinces to control the huge malaria epidemic that hit the Burundi highlands [49]. The lessons learned during this 2000 epidemic encouraged the MoH to undertake measures to improve the surveillance, the prevention and the response of future malaria outbreaks. Since 2001, a weekly data reporting selected infectious diseases, including malaria, has been set up in all health facilities. In January 2004, the MoH and WHO elaborated a national strategy to prevent, to detect earlier and to control epidemics in Burundi [50]. This plan included increased epidemiological surveillance, improved case management with artemisinine-based combination treatment (ACT) and the strengthening of human resources (increase in human resources and trainings) in the health facilities. From 2003 to 2007, Burundi received 17.8 millions USD by the Global fund for malaria. The new national anti-malarial drug policy (Artesunate-Amodiaquine) was adopted in December 2003 and the government is subsidizing the cost of these new drugs (0.2 USD) to be affordable for all. In 2006, *in vivo* tests to evaluate the efficacy of the new drugs after two years of implementation were performed in two sites. The efficacy of the combination therapy was 93.1% in the highland area of Buhiga and 97.4% in Kigobe situated in the plain near the Tanganyika Lake (Institute of Tropical Medicine, Antwerp, unpublished data). IRS was implemented to prevent future epidemic in Karuzi highland (2002-2005) [39] and other IRS campaigns were started in certain highland areas when malaria cases reach emergency threshold (Ngozi 2002). Since 2005, systematic distribution of free ITNs by the MoH to pregnant women and children less than five years has been integrated in routine health services. The non profit organization Population Services International (PSI) is selling highly subsidised ITNs via social marketing programme in nine provinces targeting the whole population. According their evaluation, the proportion of pregnant women sleeping under a net increases from 7.1% in 2005 to 28% in 2006 and from 6.5% (2005) to 26.6% (2006) for the children under five years old (data presented during the Roll Back Malaria meeting in Burundi, 2007). However this data are far from the objective of the Roll Back Malaria to reach the 80% coverage by 2010 and a 50% reduction in malaria burden [51]. The global fund has reported a drop in malaria cases of 39% since 2000 [52], but this figure should be taken with cautious and could be overestimated, 2000 being an epidemic year.

2.8. The future

Within the framework of the SUFI (Scaling Up for Impact, World Bank), a five year Roll Back Malaria strategic plan 2008-2012 has been developed and by 2010, the coverage rate for

the core malaria interventions (quick access to effective antimalarial treatment, net use and spraying) is expected to reach more than 80% of the population. Through public health facilities, ACT will be widely deployed in the private sector and at the community level through the “home management of fever strategy”. Integrated campaigns of ITN distribution are planned for 2009 with the support of the Global Fund.

However to ensure effective delivery, and follow up of these interventions, there is a need to strengthen health services, health management information system and reinforced human resources especially in the rural remote areas. Besides, some supportive strategies such as monitoring and evaluation, communication, operational research should be strengthened. The creation of a national malaria control programme is also a priority to focus available human resources for the implementation of an effective malaria control programme.

It has been calculated that Burundi should disburse between 2006 and 2010 about 18.4 million US$ by year for reaching the RBM target [53]. Burundi will receive in 2008, 33.7 million US$ for six years by the global fund and other partners as UNICEF and the World Bank are also involved in malaria control in Burundi. With strong and sustain political wills and additional fundings, the burden of malaria could be greatly reduced in Burundi especially in the highlands.

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Chapter 3. Vector control in a malaria epidemic occurring within a complex emergency situation in Burundi: A case study

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3.1. Abstract

African highlands often suffer of devastating malaria epidemics, sometimes in conjunction with complex emergencies, making their control even more difficult. In 2000, Burundian highlands experienced a large malaria outbreak at a time of civil unrest, constant insecurity and nutritional emergency. Because of suspected high resistance to the first and second line treatments, the provincial health authority and Médecins Sans Frontières (Belgium) decided to implement vector control activities in an attempt to curtail the epidemic. There are few reported interventions of this type to control malaria epidemics in complex emergency contexts. Here, decisions and actions taken to control this epidemic, their impact and the lessons learned from this experience are reported. Twenty nine hills (administrative areas) were selected in collaboration with the provincial health authorities for the vector control interventions combining indoor residual spraying with deltamethrin and insecticide-treated nets. Impact was evaluated by entomological and parasitological surveys. Almost all houses (99%) were sprayed and nets use varied between 48% and 63%. *Anopheles* indoor resting density was significantly lower in treated as compared to untreated hills, the latter taken as controls. Despite this impact on the vector, malaria prevalence was not significantly lower in treated hills except for people sleeping under a net. Indoor spraying was feasible and resulted in high coverage despite being a logistically complex intervention in the Burundian context (scattered houses and emergency situation). However, it had little impact on the prevalence of malaria infection, possibly because it was implemented after the epidemic's peak. Nevertheless, after this outbreak the Ministry of Health improved the surveillance system, changed its policy with introduction of effective drugs and implementation of vector control to prevent new malaria epidemics. In the absence of effective drugs and sufficient preparedness, present study failed to demonstrate any impact of vector control activities upon the course of a short-duration malaria epidemic. However, the experience gained lead to increased preparedness and demonstrated the feasibility of vector control measures in this specific context.

3.2. Introduction

Malaria epidemics are a growing problem in the African highlands with devastating effects on their immunologically naive population [1,2]. When occurring during complex emergency situations their control is even more difficult. According to WHO [3] "a complex emergency is a situation that affects large civilian populations with war or civil strife, food shortages and

population displacement, resulting in excess mortality and morbidity". The approach to malaria control in the acute phases of emergencies, particularly in organized refugee camps, has been established and is based on surveillance, outbreak preparedness and case management [3,4]. However, there are a variety of situations that are much more complex where the control depends strongly on the local context.

Burundi has faced an ongoing conflict since 1993. Massive movements of the population have been recorded and according to the Office for the Coordination of Humanitarian Affairs (OCHA) more than 500,000 people were internally displaced in Burundi at the end of 2000. In addition to the civil war, Burundi faced, an increase in malaria cases in the whole country and small outbreaks were recorded in two highland provinces in the late nineties [5]. From October 2000 to March 2001, a large malaria epidemic occurred in the Burundian highlands [6], with 2.9 million registered cases over a population of 6.7 million. Between 1,000 to 8,900 probable malaria deaths were reported in three highland provinces, representing between 51% to 78% of the overall mortality [7]. This epidemic was the result of a combination of different factors including land use changes, population movements, climate variability, deteriorating health systems and malnutrition, further compounded by a high level of resistance against the main drugs chloroquine (CQ) and sulphadoxine/pyrimethamine (SP).

In Karuzi, one of the highland provinces, several actions were taken in progression to contain the increasing number of malaria cases (Figure 9). First, early November 2000, the health staff was increased, a simplified malaria treatment protocol was implemented, the hospital capacity was doubled and two mobile clinics were set up, the latter with the intention of decreasing the health facilities' workload and reaching more isolated populations. Secondly, mid-November, the Ministry of Health (MoH) declared the epidemic and antimalarial drugs were provided free-of-charge. Médecins Sans Frontières Belgium (MSF-B) supplied all the public and private health facilities with CQ, SP and quinine. However, because of the suspected high CQ and SP resistance, the first and second line treatment at the time of the epidemic, the MoH in collaboration with MSF-B planned an evaluation of the resistance against these drugs. Using non efficacious drugs would not stop the epidemic and could even worsen it [8,9]. Hence, the need for an alternative strategy to control the transmission and reduce clinical malaria was required, before a new national drug policy based on the results of the resistance monitoring could be adopted.

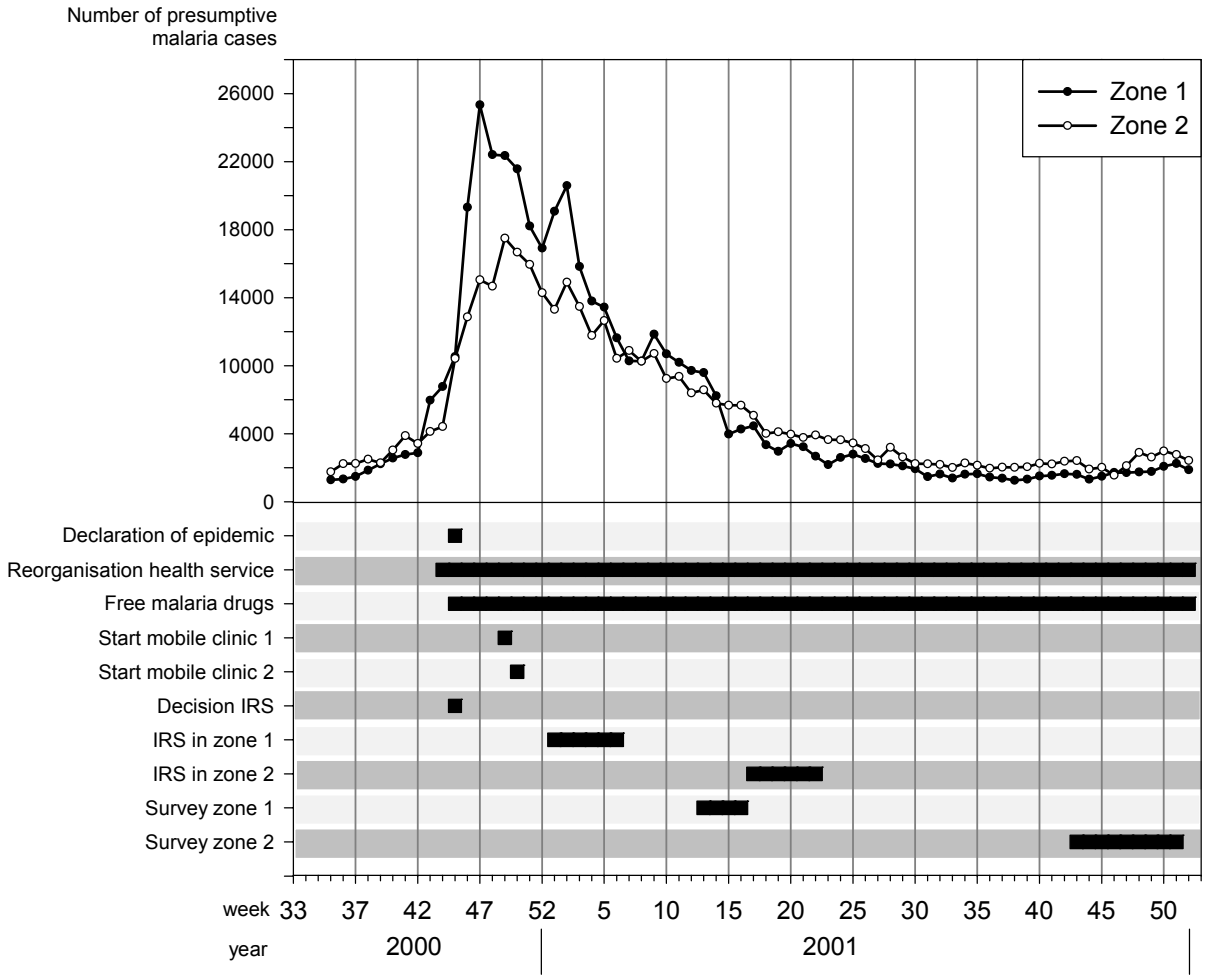

Figure 9: Overview of the malaria epidemic and control interventions in the highland of Karuzi province, Burundi**.**

Number of presumptive malaria cases recorded separately in the health centre of Zone 1 and 2 by weeks. Decisions and actions are plot according the date of their implementation.

Two additional interventions were considered. The first was indoor residual spraying (IRS), a treatment that can effectively control epidemics but usually only when implemented at an early stage of the outbreak [3]. Despite some reservations, regarding the timing of control activities, it was expected that IRS might work in this case. There is no literature describing field experiences of such an intervention to control an epidemic with conditions related to a complex emergency in the highlands. The second intervention was the use of insecticide-treated bed nets (ITN) that has been shown to reduce malaria morbidity and mortality where malaria is stable [10-12], though there is little documented evidence for the control or prevention of epidemics [13]. The malaria vectors in the Burundian highlands, *Anopheles*

funestus and *Anopheles gambiae* s.l., are highly endophilic and endophagic [14-16] so that IRS or ITN or both combined had the potential of controlling the epidemic through their impact on the mosquito population.

The objective of this case study is to report on the decisions made and the actions taken to control the 2000/2001 epidemic in Karuzi province, by vector control and to present an evaluation of the programme and the lessons learned from this experience.

3.3. Case description

3.3.1. Study area

Karuzi is a poor highland province in north-east Burundi with a population of 302,000 people at the time of the epidemic. The area is hilly with altitudes ranging between 1,400 to 1,900 metres. The valleys are fertile and humid, offering breeding sites for *An. gambiae* and *An. funestus*. The annual rainfall ranges between 800 and 1,300 mm, generally between October and April. The highest monthly mean temperatures occur between August and September (19°-20°C). The basic administrative unit is the "colline" (hill), 145 in the whole province distributed into seven communes.

3.3.2. Emergency context

In Burundi, there has been a civil war since 1993. Hundred thousands of people were internally displaced or crossed the Tanzanian border. An international economic embargo further impoverished the population. Since the beginning of the conflict, and until 2000, the complex emergency, on the background of general insecurity, was characterized by displaced people, a collapsing health system, environmental deterioration and poor housing conditions. In addition, the famine that occurred in Karuzi at the end of 2000, because of the drought and poor harvest, resulted in dramatic increase of malnourished cases. A nutritional survey in November 2000 reported that 24% of the population was acutely malnourished (MSF-B unpublished data). In Karuzi, a retrospective mortality survey from November 2000 to March 2001 reported a crude mortality rate of 1.1/10,000/day, an under-five mortality rate of 3.0/10,000/day which is far above the emergency threshold of 2.0/10,000/day [7].

MSF-B started to work in Karuzi in 1993 by opening a medical emergency programme providing assistance to the local population and supporting the public health services. By mid-October 2000, the number of malaria cases in the health centres doubled over one week, a clear sign that an epidemic was beginning. In just two weeks, malaria cases increased from 17,000 to 43,330. The epidemic peaked in December (Figure 9), with a 10-fold increase of cases reported by the health centres as compared to the previous three years. The weekly number of cases remained at around 30,000 throughout January and slowly decreased the following months to return to "normal" values in May 2001.

3.3.3. Vector control interventions

The vector control activities were carried out in collaboration with the Transmissible and Deficiency Disease Control Programme (LMTC) and the Provincial Health Office. Despite the decision to implement vector control measures, it was impossible to cover the whole province and intervention areas had to be chosen on the basis of the malaria burden. Unfortunately, the information available was not reliable; health services were so disorganized that the patients' origin was no longer recorded and, hence, a list of the most affected areas was unavailable. Therefore, 29 hills (4-5/communes) were selected (Figure 10), regardless of more specific criteria, based on anecdotal evidence given by provincial authorities and because of insecurity in other areas.

In each commune, 14 teams (six people each) of local inhabitants were trained on IRS, following the recommended application procedure defined by Lacarin and Reed [17]. Deltamethrine 2.5WP (K-Othrine) was applied at the target dose of 0.025g a.i./m². Each person would spray 10 houses by day. The team supervisor checked the quality of the spraying procedure and collected information on the insecticide used, the characteristics of the house and the corresponding number of people. Between December and January all health facilities, feeding centres and boarding schools were sprayed and provided with ITNs. The rest of the intervention started during the second week of January in the targeted hills of Buhiga, Bugenyuzi and Gitaramuka (Figures 9 and 10), called zone 1. The communes of Gihogazi, Mutumba, Nyabikere and Shombo were treated between April and June 2001 because of a delay in obtaining the insecticide. These communes were called zone 2 (Figures 9 and 10).

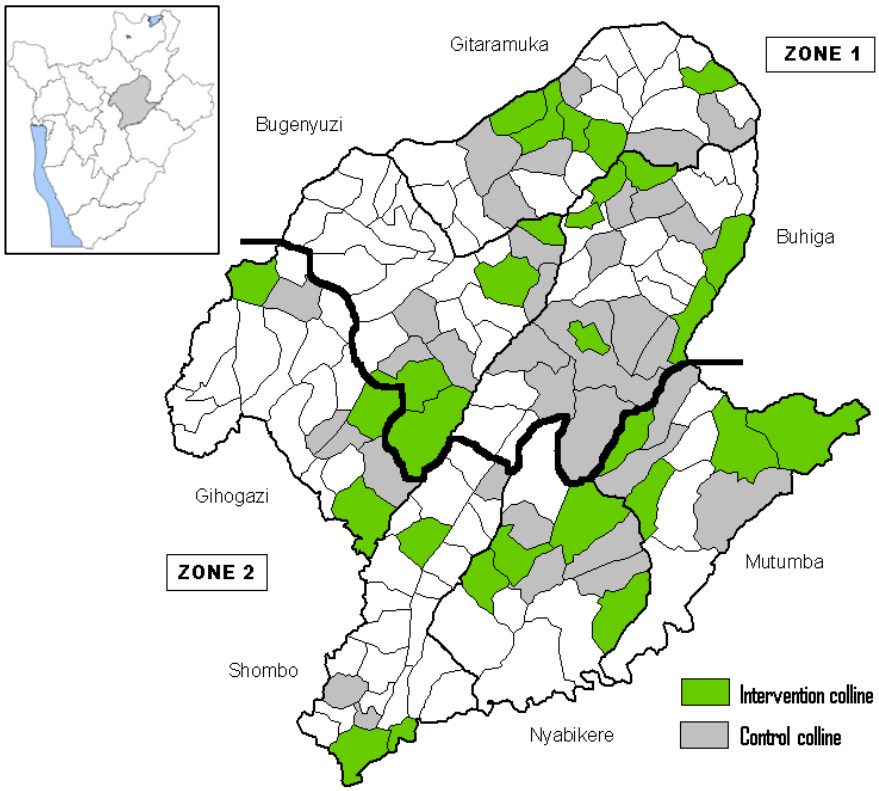

Figure 10: Map of Karuzi province showing the intervention (treated hills) and control hills.
The Karuzi province is composed of "colline" (hills), represented by small polygons and regrouped in 7 communes (Buhiga, Bugenyuzi, Gihogazi, Gitaramuka, Nyabikere, Mutumba and Shombo). The green polygons corresponded to the targeted hills for the vector control and grey are the hills selected to be the control areas for the survey. The two zones (Zone 1: survey done in March-April 2001 two months after the intervention. Zone 2: survey done from October to December 2001, five months after the intervention) are separated by a thick black line.

Each sprayman treated an average of 7.7 houses per day (Table 2), less than the planned target of 10 houses by day based on grouped camps or villages. Supervision was difficult due to the dispersion of the houses, the hilly environment and the absence of roads. At least once a week, some areas could not be reached because insecurity and this resulted in a delay of the supply of insecticide. Despite these problems and thanks to the good collaboration of the community, most houses (16494/16616; 99.3%) were covered by IRS (Table 2). On every intervention hill, an educational campaign for ITN was implemented before the distribution of one ITN (Permanet® first generation) by household. A total of 16,781 ITNs were distributed (Table 2). In zone 2, most houses (91.8%; 95% CI: 83.8-96.6), had at least one ITN (installed or not) while this percentage was lower in zone 1 (61.2%; 95% CI: 50.0-71.6). However, the

number of installed ITN was not significantly different in the two zones (zone 1: 78.8%; 95% CI: 65.3-88.9, zone 2: 69.2%; 95% CI: 57.8-79.2; P=0.2).

Table 2: Result of the vector control activities by zone

Indicators	Zone 1	Zone 2
No. of inhabitants in the province	151,563	150,299
No. of inhabitants protected by IRS	32,450	36,457
% of inhabitants protected by IRS in the province	21.4%	24.3%
No. of houses in the target hills	8,853	7,763
No. of houses sprayed in the target hills (%)	8,758 (98.9%)	7,736 (99.7%)
No. of households sprayed per man/day	8.7	6.9
No. of mosquito nets distributed	8,853	7,928

3.3.4. Parasitological and entomological survey

Survey design

Considering the emergency context no baseline survey before the vector control interventions was planned. In zone 1, a survey was carried out from 26 March to 21 April 2001 and in zone 2 from 22 October to 19 December 2001, or respectively two and five months after the end of the IRS (Figure 9). The survey includes all intervention hills. For each intervention hill, the nearest hill with the closest number of inhabitants was included as control hill (Figure 10). In each zone, the total number of houses to be selected was 85 in intervention hills and 85 in the thirty five selected control hills. The number of houses to be sampled by hill was calculated according the population density of every hill. Then from a list given by the local administration of the hill, houses were selected at random.

Daytime indoor resting mosquitoes were collected using the spray collection method [18]. After having spread white sheets on floor, the house was sprayed inside with pyrethrum, a non residual insecticide. The mosquitoes falling on the white sheets were collected and morphologically identified to species using M.T. Gillies's keys [19].

In each house, where the spray catches were done, one inhabitant was randomly selected and a rapid diagnostic test (RDT, Paracheck®) to detect *Plasmodium falciparum* specific antigens, was performed. People with a positive RDT were treated with oral quinine (10mg/kg/day x 3 during seven days). Additional information on living conditions, past malaria history and treatment was also collected.

Participating individuals were informed of the objectives of the study and verbal consent was obtained. This study was a programme evaluation and was carried out with full cooperation and approval of the Burundi Ministry of Health and the Karuzi provincial authority. It was also reviewed and approved by the MSF Ethics Committee.

Data analysis

Data were entered into MS Excel and analysed using Epi Info version 3.3.2 (Centers for Disease Control and Prevention, Atlanta). Descriptive statistics were used to summarize demography data. Chi squared analysis was used to compare the proportions. Bivariate analyses were performed to see the relative protective effect of IRS and ITN to the outcomes using a negative binomial regression for the *Anopheles* indoor resting density and a logistic regression for the malaria prevalence (Stata intercooled version Nine). Density ratios (DR = exponential of the regression coefficient) and odds ratios (OR) are reported.

3.4. Results

Characteristics of the study population and selected houses are summarized in Table 3 and were similar for control and intervention hills in the same zone. In the intervention hills of zones 1 and 2 respectively, 34.1% and 44.7% of the selected persons declared having slept under a net the previous night, whereas in control areas only one person out of 170 did so. In each zone, the spray catches were done in the 170 selected households (85 in the intervention hills and 85 in the controls). In zone 1, the majority of *Anopheles* (95.2%) was *An. gambiae* s.l., the remaining being *An. funestus* while in zone 2 both species were present in almost equal proportions (*An. gambiae* s.l.: 45.1%; *An. funestus*: 54.9%). In zone 1, the protective effect of IRS against *Anopheles* in treated houses was 95% (95% CI: 80-99) compared to control houses and adjusted for net use, in zone 2, it reached 87% (95% CI: 31-98) (Table 4). Using a net was not followed by a significant reduction of *Anopheles* indoor resting density (Table 4). No difference in malaria infection was found between sprayed and non-sprayed hills whereas in zone 1, prevalence was lower in people sleeping under a net (Table 5). The difference in prevalence detected between the two intervention zones (zone 1: 60%, zone 2: 30%) is probably due to the natural decline of the epidemic as survey in zone 2 was carried out several months after the survey in zone 1 (Figure 9). Moreover, the proportion of persons reporting a malaria attack during the past two months was similar between control and

intervention hills but was lower in October December (zone 2: 37.1%) compared to the period of March-April (zone 1: 77.1%) (Table3).

Table 3: Characteristics of the study population and houses by areas (intervention hills, control hills) and by zones

	Zone 1		Zone 2	
	Intervention hills	Control hills	Intervention hills	Control hills
Study population	n=85	n=85	n=85	n=85
Median age in year (percentile 25-75)	19 (9-38)	20 (9-40)	18 (7-32)	20 (7-37)
Proportion of women	58.8% [a]	58.8% [a]	62.4% [a]	52.9% [a]
At least one malaria attack during the last 2 months	74.1% [a]	81.2% [a]	35.3% [b]	38.8% [b]
At least one malaria treatment the last 2 months	52.9% [a]	64.7% [a]	7.1% [b]	8.2% [b]
Houses	n=85	n=85	n=85	n=85
Traditional houses[1]	92.9% [a]	90.6% [a]	95.3% [a]	95.3% [a]
Roof made of thatch	56.4% [a]	69.4% [a]	49.4% [a]	41.1% [a]
Open eaves	42.4% [a c]	52.9% [a]	29.4% [b c]	20.0% [b]
Animals inside	37.6% [a]	42.4% [a]	68.2% [b]	67.1% [b]
Houses near the marsh[2]	28.2% [a]	29.4% [a]	47.1% [a b]	57.6% [b]

[1] Walls make with mud bricks or mud, [2] Houses within 500 meters
Results on the same line with identical subscript letter are not significantly different

Table 4: Impact of spraying and net use on *Anopheles* indoor resting density by zone using a multivariate negative binomial regression.

	Adjusted DR*	95% CI	P value
Survey Zone 1			
Sprayed vs not	0.05	(0.01-0.20)	<0.001
Net used vs not	0.47	(0.06-3.65)	0.470
Survey Zone 2			
Sprayed vs not	0.13	(0.02-0.69)	0.017
Net used vs not	0.96	(0.14-6.58)	0.964

* Density Ratio = exponential of the regression coefficient adjusted for net use and spraying, CI = abbreviation for Confidence Interval

Table 5: Impact of spraying and sleeping under net on malaria prevalence by zone using a multivariate logistic regression.

	Prevalence % (N)	Adjusted OR*	95% CI	P value
Survey Zone 1				
Spraying				
Yes	60.0% (85)	1.65	(0.82-3.32)	0.160
No	56.5% (85)	1.0		
Sleeping under net				
Yes	43.3% (30)	0.36	(0.15-0.88)	0.026
No	61.4% (140)	1.0		
Survey Zone 2				
Spraying				
Yes	28.2% (85)	0.74	(0.34-1.61)	0.446
No	34.1% (85)	1.0		
Sleeping under net				
Yes	29.0% (38)	1.07	(0.41-2.75)	0.896
No	31.8% (132)	1.0		

*Odd ratio adjusted for sleeping under net and spraying

3.5. Discussion

Despite the difficulties encountered, a vector control programme based on IRS and ITN was feasible in an open setting associated with a complex emergency situation. Excellent coverage was obtained for IRS and moderately good coverage for ITN.

Ideally un-treated sentinel houses should have been chosen to evaluate the mass effect of IRS on the vector population. In present study, vector density was estimated in treated houses providing an evaluation of the treatment status of the houses. However the endophillic behaviour of *Anopheles* is very pronounced in the highlands of Burundi [15] probably restricting the resting sites in houses or shelters where the average temperatures are 3 to 5°C above the outside temperatures [16,20]. Furthermore, more than 99% of the households were sprayed including the cattle sheds and separate kitchens. It can then be assumed that the used collection method provides also a representative picture of the vector density.

IRS reduced drastically the *Anopheles* indoor resting density, although the prevalence of malaria infection did not follow accordingly. However, sleeping under a net reduced the prevalence of 64% in zone 1 whereas no difference was seen in zone 2. The absence of impact

of the ITN in zone 2 can be explained by the end of the transmission period and the natural decrease in prevalence in both intervention and control hills so that no potential protective effect of the net could be seen.

The malaria cases as reported by the health centres (Figure 9) started to decline during the vector control intervention in zone 1, which could hardly be explained by the intervention itself. In zone 2 the cases reached the pre-epidemic level before the intervention. Moreover, although observed in two different control zones, malaria attacks reported during the October-December survey was half of that observed during the March April survey. Both observations suggest that the decline of the malaria incidence was mainly natural and there is no evidence that vector control activities may have sped up the resolution of the epidemic. It was mentioned earlier that IRS is useful only if applied in a timely manner at the start of the epidemic and has little or no impact on malaria epidemics if implemented when peak is reached [3]. In Burundi, the malaria epidemic was recognized late because, after 10 years of civil war, the health services were unprepared for it. Surveillance, outbreak preparedness and responses were not well developed [6]. In addition, vector control activities were started only two months after the decision had been taken despite the availability of the expertise and equipment at the LMTC. This could be explained by an underestimation of the required time and equipment due to poor information on vector control strategies in open settings, the difficulties of establishing the areas most affected and the chronic insecurity in the province which delayed the beginning of the intervention. However, vector control activities were started because good case management could not be achieved due to presumptive poor efficacy of CQ and SP. The *in vivo* resistance tests carried out afterwards reported a failure by day 14 of 93% for CQ and 66% for SP (MSF-B, internal report). These results prompted the MoH to recommend an interim drug policy with SP as a first line drug and artemether-lumefantrine to be used during malaria epidemics. The final drug policy with amodiaquine-artesunate as first line treatment was implemented at the end of 2003 [21].

The lessons learned during the 2000 epidemic encouraged the MoH to undertake measures to improve the surveillance, the response and the prevention of future malaria outbreaks. Since 2001, a weekly collection of some infectious diseases, including malaria, has been set up in all health facilities. In January 2004, the MoH and WHO elaborated a national strategy [22] to prevent, to detect earlier and to control epidemics in Burundi. This plan included, increased epidemiological surveillance, improved case management with artemisinine-based

combination treatment (ACT), the strengthening of human resources in the health facilities, the distribution of mosquito nets and focal IRS in areas most at risk. Since 2005, systematic distribution of long lasting mosquito nets to pregnant women and children under five years has been integrated within routine health services. Indeed, the target groups are provided with ITN through the first antenatal cares and measles vaccination. Furthermore, acquired experience at the provincial and national level on vector control will be useful for future activities and could, with improved epidemic preparedness, greatly reduce the risk of recurrent epidemics.

Since 2001, some highland provinces were affected by higher number of malaria cases, reaching emergency thresholds in 2002 and 2005 (MoH data). However these increases were limited in time and confined to smaller areas than the 2001 epidemic. The implementation of more systematic vector control activities could be one of the reasons for the absence of true epidemics. Furthermore the introduction of ACT in December 2003 could have reduced the malaria transmission as reported in low endemic areas [23,24]. The possible acquisition of a protective immunity as observed in the Kenyan highlands population [25] could even play a more important role to explain the absence of epidemics. In Karuzi, from 2002 to 2006 a change in endemicity was observed compared to the 1998 classification of the MoH with prevalence reaching 35 to 50% in age group of two to nine years old and with a high proportion of asymptomatic carriers recorded (unpublished data).

Vector control measures based on IRS and ITN may be more appropriate for the prevention of malaria epidemics in the highlands [26,27]. One round of IRS, before the transmission period and targeted to areas near the valley marshes, could reduce the vector population, the intensity of transmission levels and the human reservoir, hence, the risk of a devastating epidemic.

In the absence of effective drugs during an epidemic of malaria in the highlands of Burundi, vector control programme combining IRS and ITN was feasible despite a context of complex emergency. Vector populations were much reduced, but there is no evidence that the vector control intervention changed the natural evolution of the epidemic. This programme did, however, lead to better surveillance systems being established by the government so that future epidemics may be identified earlier. As well, the experience gained from the IRS and ITNs showed that these measures, known to be effective in preventing epidemics, could be feasibly introduced, even in the context of a complex emergency situation. The combination

of improved prevention, earlier detection, and treatment with more effective drugs should help to make serious epidemics of malaria in the Burundi highlands a thing of the past.

3.6. Authors' contributions

NP collected and analysed the data and drafted the manuscript. MC and WVB provided crucial inputs in the data analysis and the writing up of the manuscript. UD and TR improved the manuscript. MVH designed the survey and with, PM and DB facilitated the data collection and gave technical advice on the manuscript. All authors read and approved the final manuscript.

3.7. Acknowledgements

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Chapter 4. Spatial targeted vector control in the highlands of Burundi and its impact on malaria transmission

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4.1. Abstract

Prevention of malaria epidemics is a priority for African countries. The 2000 malaria epidemic in Burundi prompted the government to implement measures for preventing future outbreaks. Case management with artemisinin-based combination therapy and malaria surveillance were nationally improved. A vector control programme was initiated in one of the most affected highland provinces. The focal distribution of malaria vectors in the highlands was the starting point for designing a targeted vector control strategy. The objective of this study is to present the results of this strategy on malaria transmission in an African highland region. In Karuzi, in 2002-2005, vector control activities combining indoor residual spraying and long-lasting insecticidal nets were implemented. The interventions were done before the expected malaria transmission period and targeted the valleys between hills, with the expectation that this would also protect the populations living at higher altitudes. The impact on the *Anopheles* population and on malaria transmission was determined by nine cross-sectional surveys carried out at regular intervals throughout the study period. *Anopheles gambiae* s.l. and *Anopheles funestus* represented 95% of the collected anopheline species. In the valleys, where the vector control activities were implemented, *Anopheles* density was reduced by 82% (95% CI: 69-90). Similarly, transmission was decreased by 90% (95% CI: 63-97, $p = 0.001$). In the sprayed valleys, *Anopheles* density was further reduced by 79.5% (95% CI: 51.7-91.3, $p < 0.001$) in the houses with nets as compared to houses without them. No significant impact on vector density and malaria transmission was observed in the hill tops. However, the intervention focused on the high risk areas near the valley floor, where 93% of the vectors are found and 90% of the transmission occurs. Spatial targeted vector control effectively reduced *Anopheles* density and transmission in this highland district. Bed nets have an additional effect on *Anopheles* density though this did not translate in an additional impact on transmission. Though no impact was observed in the hilltops, the programme successfully covered the areas most at risk. Such a targeted strategy could prevent the emergence and spread of an epidemic from these high risk foci.

4.2. Introduction

Malaria epidemics occur frequently in the African highlands [1-3]. Their control is a priority and a specific plan of action was adopted by the African leaders during the 2000 Abuja summit [4]. An early warning system to increase malaria epidemic preparedness and prevention has been promoted, based on climate data, population vulnerability indicators,

environmental factors and disease surveillance [5]. Models proposed seems reliable in desert fringes [6,7], where rainfall is the main driving factor of epidemics [8]. However, the available forecasting models may not be accurate enough for the African highlands where most populations at risk of epidemics reside [8,9]. Consequently, in the highlands, routine implementation of preventive measures and prompt response to an unexpected increase of malaria cases are the main components for the control of epidemics.

In the last decade, Burundi has faced an increase in malaria cases with a major malaria outbreak in 2001 [10]. To contain the epidemic, Indoor Residual Spraying (IRS) and Long-Lasting Insecticidal Nets (LNs) have been implemented in the highland province of Karuzi [11]. Due to its late implementation, this strategy was unable to have any impact on the epidemic. However, this experience showed that these interventions were feasible, even in the context of a complex emergency situation.

Following the 2001 epidemic, the national health authorities decided to improve data collection, adopted an interim treatment protocol based on artemether-lumefantrin only during malaria epidemics and started studies to change the national treatment policy for an artemisinin-based combination therapy (ACT). Furthermore, in Karuzi, one of the most affected areas, focal vector control activities were implemented. The objective of these measures was to prevent future malaria outbreaks.

In epidemic areas, the distribution of anopheline mosquitoes and malaria transmission are usually focal [12] and often negatively associated with distance from rivers or valley bottoms [13,14]. Therefore, rather than implementing vector control activities over large areas, it was felt that they could be targeted to places where most malaria transmission occurs, possibly reducing the implementation costs, and enhancing their sustainability without losing effectiveness [15]. Besides reducing transmission in the targeted valleys, it was thought that the hills above the IRS areas would also benefit as they would be shielded from the transmission occurring from below [16]. Similar approach, i.e. focal intervention based on vector behaviour, was successfully used to control malaria with environmental measures in the early 20th century in Indonesia [17] but later abandoned in the DDT era. Since then, only one study addressed this issue for African highland [16]. This paper reports the results of such targeted intervention on vector density and malaria transmission in the Burundi highlands.

4.3. Methods

4.3.1. Study area

Karuzi is a central highland province in Burundi. A detailed description was presented elsewhere [11]. In 2002, malaria was the main cause of morbidity, representing 57% of all attendance to health facilities (Médecins Sans Frontières-Belgium malaria dataset, 2002). Malaria cases peak in June-July and in November-December. The recent epidemics in the Burundian highlands were mostly recorded towards the end of the year (EPISTAT: Epidemiology and Statistic Cell, Ministry of Health, Burundi).

4.3.2. Interventions and study

Between 2002 and 2005, an annual IRS round (June-July) was carried out targeting the areas at the foot of the hills before the second transmission period. The rationale of such choice was based on the observation that malaria vectors seldom spread further than one kilometre radius from the breeding sites [15,17]. In the highlands, vectors are usually clustered at the valleys' bottom from where they do not spread beyond 500 meters [18]. In this study only the valleys, i.e. the zone from the river/marsh at the bottom of the valley up to 700 meters uphill, were treated, while the upper part of the hills were left untreated. In the intervention areas, IRS was carried out in all human dwellings (interior walls and ceilings) and cattle sheds with the residual insecticides deltamethrin 5 WP (in 2002-2004) or alphacypermethrin 5 WP (in 2005) at the dose of 25mg a.i./m². In 2002, the LN (PermaNet® 1.0) distribution preceded the first IRS round and consisted of two nets for each sprayed house.

The larger cultivated valleys, with the highest population density, were chosen for the intervention, while other areas were identified as control areas (Figure 11). Because intervention areas were actually selected for their higher malaria risk and this for obvious ethical reasons, they are probably not entirely comparable to control areas. Antimalarial treatment was available for both intervention and control areas. The total length of both sides of the valley floors, control (74 kilometres) and intervention (331 kilometres) alike were equally divided into 100 points on a digital map of the province. For each survey, 25 points were randomly selected for both intervention and control area and their latitude and longitude sent to a hand-held global positioning system (GPS 76, Garmin®). From the geographical location of each selected point, two clusters consisting of either four or eight houses (according to the survey) were chosen; The valley clusters comprised houses located around a

randomly chosen point on a vertical line running between 100 and 600 meters from the bottom; The hilltop clusters comprised houses located around a randomly chosen point on a vertical line running between 100 and 600 meters from the "limit" (700 metres from the valley bottom) separating valley and hill top (Figure 12). Hence, four zones were identified: (1) the intervention valleys with treated houses, (2) the corresponding intervention hill tops without treatment, (3) the untreated control valleys and (4) the untreated control hill tops. In total 4 x 25 clusters were re-sampled for each survey.

A baseline entomological survey (Survey 1) was done before the intervention and was followed for four years by two annual surveys: one three months (November-December: survey 2, 4, 6, 8) and the other nine months (April-May: survey 3, 5, 7, 9) after the yearly IRS. The types of houses (size, open eaves, walls and roof), presence of animals, location of the kitchen, altitude and distance from the selected cluster to the swamp were recorded, based on direct observation.

Daytime indoor resting mosquitoes were collected by spray-sheet catches using aerosol with pyrethrum and piperonyl butoxide [19]. These spray-sheet collections were done in randomly selected un-sprayed houses of the control areas and intervention hill tops. In the intervention valleys the collection was made regardless of the spraying status of the houses during the preceding IRS round. The Indoor Resting Density (IRD) was determined as the average number of *Anopheles* collected for each house.

Anophelines were morphologically identified and classified as *Anopheles gambiae* sensu lato (s.l.) and *Anopheles funestus* using a simplified key adapted from Gillies [20]. The feeding status (unfed, blood-fed, half-gravid and fully-gravid) was also scored. A sample of *An. gambiae* s.l. and *An. funestus* mosquitoes was analysed by species specific Polymerase Chain Reaction (PCR) [21,22]. The head and thorax of all collected females were individually tested with an enzyme-linked immunosorbent assay (ELISA) adapted from Wirtz [23] for the presence of *Plasmodium falciparum* circumsporozoite antigen. The sporozoite rate (SR) was computed as the proportion of ELISA positive mosquitoes. The number of infective bites per house per month was estimated as the number of fed *Anopheles* resting indoor and positive for *P. falciparum* by the ELISA test [24,25].

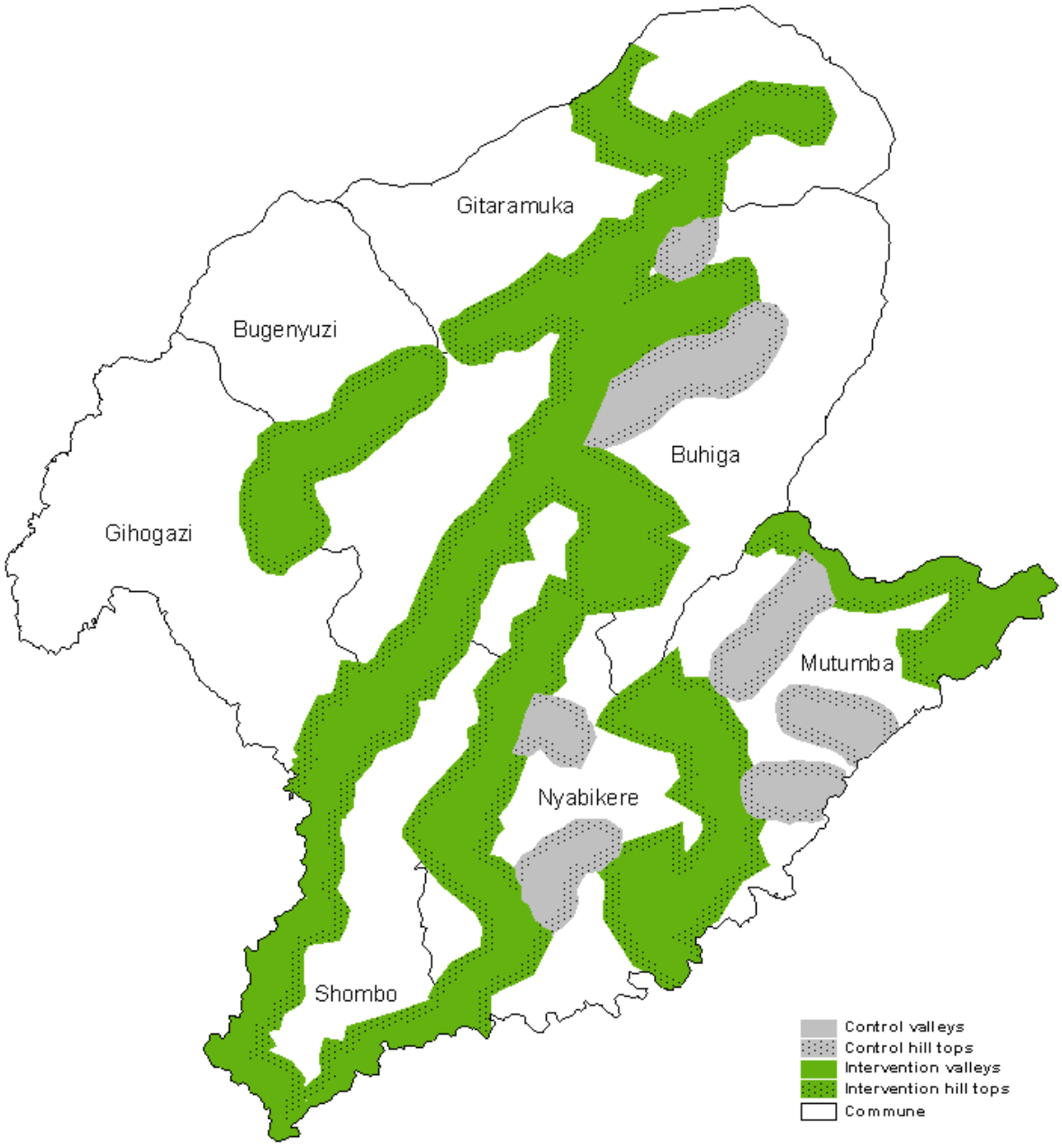

Figure 11: Map of Karuzi Province (Burundi) showing the intervention and control areas.
In the intervention areas only the valleys were sprayed depicted in green. The hill tops were not sprayed (dotted green).The control areas are represented in grey for the valleys and dotted grey for the hill tops.

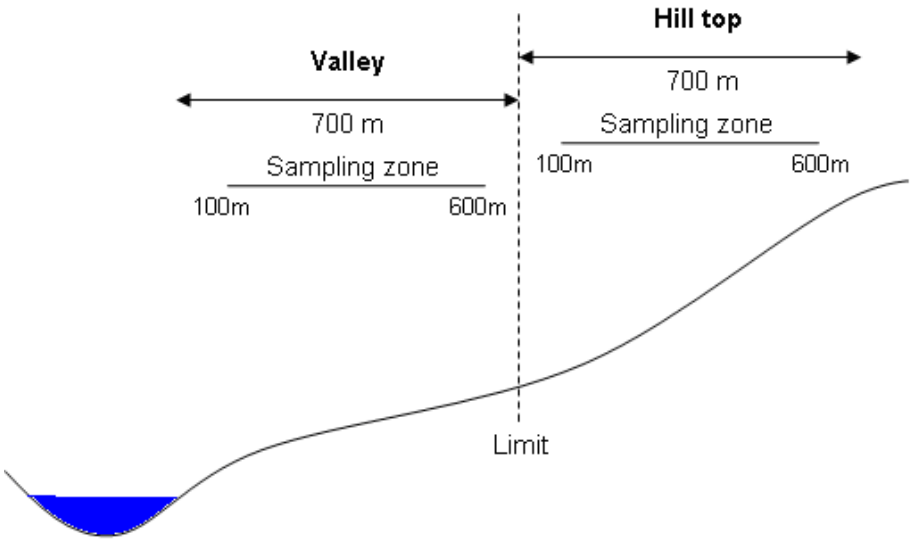

Figure 12: Representation of the valley and hill top areas, showing the sampling zones.
From the valley floor, clusters in the valley where chosen at random between 100 and 600 metres. Clusters in the hill top were selected from 100 to 600 metres from the limit separating valley and hill top.

4.3.3. Statistical analysis

Data were analysed using STATA software (Stata-Corporation, USA, version 9.2). In all statistics analyses, house clusters were taken into account to calculate robust standard errors and 95% confidence interval (95% CI). Logistic regressions were used to analyse the SR whereas negative binomial regressions were used for the counts (IRD and number of infective bites).

Baseline data on housing characteristics in the four zones were summarized by means of proportions or means. All entomological indicators, *Anopheles* species, *An. funestus* and *An. gambiae* IRD, SR and number of infective bites were analysed separately for the valleys and the hilltops data. *Anopheles* IRD was tested using the survey identification, intervention vs. control and the interaction between both as discrete explanatory variables. The density of *An. funestus* and *An. gambiae* in the hilltops were also computed using the seasons (before, three months or nine months after IRS), the intervention vs. control and their interaction terms as explanatory variables. SR and the number of infective bites were analysed for the dataset of surveys 2-9 pooled together with intervention vs. control as explanatory variables.

All entomological indicators were analysed in control areas for surveys 2-9 using hilltop vs. valley as only explanatory variable. Finally, the effect of the LNs on the *Anopheles* IRD and on the number of infective bites was evaluated on the dataset restricted to the intervention valleys. Density Ratios (DR) were calculated as the exponential of the negative binomial regression coefficient for the IRD and for the number of infective bites.

4.3.4. Ethical issues

Verbal consent was asked to the head of each household for the spray catches. In case of refusal (usually less than 10 by surveys) the next household was asked for permission. The vector control programme and the study were approved by the Ministry of Health of Burundi. Ethical approval for this study was granted by the Ethical Committee of the Institute of Tropical Medicine in Antwerp.

4.4. Results

4.4.1. Vector control activities

A total of 24,000 LNs were distributed (Table 6). In 2002, just after the main distribution, most nets were in use (18792/23850, 78.8%), the rest being either not used (2632/23850, 11.0%) or missing. However, during the period 2002-2005, LN use decreased to 31.2%. IRS coverage exceeded 90%, except for the year 2002. During the first IRS round, 1,600 houses in the southern part of the province could not be sprayed because of security problems. These houses were treated and received LNs the following years.

Table 6: Coverage of the vector control activities, Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Net (LN) by year

	2002	2003	2004	2005
Total provincial population*	302 062	311 134	320 458	329 431
No. of targeted houses (%treated)	14 783 (86%)	15 106 (95%)	17 954 (93%)	18 072 (94%)
No. of houses treated /man/day	6.0	5.7	5.7	5.4
Insecticide used	Deltamethrin	Deltamethrin	Deltamethrin	Alpha cypermethrin
No. of LN distributed	20 750	3 200	0	0
No. of net used (%**)	18 792 (78.8%)	17 631 (65.2%)	14 442 (53.4%)	8 431 (31.2%)

* Official data (EPISTAT Burundi)
** % = net used/ (LNs distributed + 3100 net present in the houses before the intervention)

4.4.2. Baseline characteristics of households and malaria transmission

Before the intervention, i.e. survey 1, the house characteristics between intervention and control areas were similar (Table 7), except for the clusters in the hill tops of the intervention areas which were more distant from the valley bottom (1,216 metres) than those in the control areas (945 metres). The separation between valleys and hill tops was chosen at 700 meters from the valley bottom. However, in the intervention areas during the houses census, the limit was moved further away in some areas and explained the difference between clusters in the hill tops. In the subsequent surveys, no major differences between control and intervention areas in terms of house type (size, open eaves, walls and roof), domestic animals, location of the kitchen, mean altitude of the clusters and distance from valley clusters to valley bottom could be found.

Table 7: Environmental and household characteristics in the intervention (I) and control (C) areas for pre-intervention survey (survey 1).

	Valleys		Hill tops	
	C	I	C	I
No. of houses sampled	100	100	99	150
Houses with animals inside	68.0 (4.5)	62.0 (6.1)	69.4 (5.1)	64.7 (6.0)
Open eaves	57.0 (5.1)	49.0 (6.0)	48.5 (6.0)	47.3 (5.7)
Separate kitchen	22.0 (4.4)	19.0 (4.6)	20.2 (4.8)	22.0 (6.0)
Size of houses				
< 25 m^2	11.0 (4.6)	28.0 (5.6)	19.2 (5.5)	19.3 (3.7)
25-50 m^2	19.0 (4.6)	22.0 (5.3)	21.2 (4.3)	34.0 (4.2)
> 50 m^2	70.0 (6.5)	50.0 (7.6)	59.6 (6.5)	46.7 (5.5)
Type of walls				
Thatch	2.0 (1.4)	10.0 (3.8)	8.1 (3.8)	8.0 (2.7)
Mud	69.0 (7.0)	54.0 (7.5)	67.7 (6.7)	61.3 (6.8)
Bricks	24.0 (6.0)	33.0 (6.6)	19.2 (4.2)	25.3 (5.4)
Other	5.0 (2.0)	3.0 (1.7)	5.1(2.1)	5.3 (2.5)
Type of roofs				
Thatch	51.0 (7.1)	53.0 (8.5)	52.5 (6.2)	55.3 (7.1)
Tile	14.0 (4.6)	16.0 (5.4)	19.2 (5.2)	12.0 (3.7)
Corrugate	19.0 (5.1)	22.0 (6.2)	23.2 (4.4)	27.3 (6.5)
Other	16.0 (4.8)	9.0 (3.2)	5.1 (2.9)	5.3 (1.9)
Altitude clusters (m)	1554 (11.9)	1548 (12.0)	1599 (18.4)	1607 (14.3)
Distance clusters/valley floors (m)	387(27.5)	404 (29.0)	945 (39.8)	1216 (71.5)

Proportions (standard error) are reported except for altitude and distance where arithmetic means (standard error) are given.

Before the intervention, malaria transmission in the valleys was significantly higher in the intervention than in the control valleys, mainly because of differences in *Anopheles* density

and not sporozoite rates (Table 8). However, the hilltops of both intervention and control areas were comparable in term of transmission and *Anopheles* density.

Table 8: Baseline Indoor Resting Density (IRD), Sporozoite Rate (SR) and infective bites (IB) as observed during the pre-intervention survey in Control (C) and Intervention (I) areas.

	Valleys				Hill tops			
	C	I	Ratio* (95% CI)	P value	C	I	Ratio* (95% CI)	P value
Total *Anopheles*								
IRD /house	1.3	7.6	5.9 (1.7-21.0)	0.007	1.3	0.6	0.4 (0.1-1.6)	0.200
SR (No.)	3.8% (104)	4.2% (737)	1.1 (0.4-3.1)	0.858	0.9% (114)	6.3% (64)	7.5 (2.2-26.2)	0.002
Fed *Anopheles*								
IRD /house	0.5	4.3	8.7 (2.3-32.9)	0.002	0.4	0.2	0.6 (0.2-1.8)	0.333
SR (No.)	2.0% (49)	4.1% (419)	2.0 (0.4-11.0)	0.397	2.8% (36)	9.4% (32)	3.6 (0.6-22.3)	0.156
IB /house/mth	0.3	5.1	17 (1.7-171)	0.015	0.3	0.6	2.0 (0.2-23.3)	0.580

*Density ratios for indoor resting density and infective bites. Odd ratios for sporozoite rates

4.4.3. Entomological results

Species composition. A total of 18,764 mosquitoes were collected indoors, 77.1% (14,474) anophelines. *Anopheles gambiae* s.l. and *An. funestus* were the most abundant species (up to 95% of catches), with females *An. gambiae* s.l. (9473, 79.3%) more prevalent than *An. funestus* (2471, 20.7%), except for survey 1 where 57.6% of *Anopheles* were *An. funestus*. *Anopheles gambiae* s.s. (98.2%) was the dominant species of the complex. A few *Anopheles arabiensis* (60, 1.8%) were collected, most of them in April-May 2004 and 2005. Within the *Anopheles* species morphologically identified as *An. funestus* (n=1898), 79.3% were *An. funestus* s.s. by species specific PCR [22]. For the remaining samples, the PCR and the sequencing analysis of the ITS2 region revealed no link with recorded species. After careful morphological identification, they could be identified as *Anopheles demeilloni* (Ralph Harbach personal communication), for which no sequence exists. This species is morphologically close to the *An. funestus* group and could not be separated using simplified identification keys. It will be further defined as "*Anopheles funestus*-like".

Indoor resting density. After the intervention, the overall reduction of *Anopheles* density in the valleys was 82.5% (95% CI: 69.4-90.0, p < 0.001) in the intervention compared to control areas. This significant difference was observed for every survey done three or nine months after IRS (Table 9). After the intervention, *Anopheles* density in the hilltops was only significantly reduced in the intervention for surveys 5 and 6. A lower density of *An. funestus* was observed for the surveys done three months after the spraying (DR: 0.45, 95% CI: 0.25-0.81, p = 0.008), while for the others the difference was not statistically significant (DR: 0.66, p = 0.199). A similar DR was observed in *An. gambiae* s.l. (DR: 0.46, 95% CI: 0.15-1.41, p = 0.174) three months after IRS. However, the high intraclass correlation of the latter data caused an important design effect (Deff = 4.3 compared to 1.7 obtained in the analysis of *An. funestus* data) and reduced the power of the statistical analysis.

Table 9: Mean indoor resting density per house of all *Anopheles* in valleys and hill tops of intervention (I) and control (C) areas. Differences by survey were tested with the negative binomial regression.

Survey	Valleys				Hill tops			
	C	I*	Density ratio (95% CI)	P value	C	I*	Density ratio (95% CI)	P value
2	3.26	0.13	0.04 (0.01-0.13)	<0.001	1.03	0.39	0.37 (0.13-1.05)	0.061
3	1.81	0.27	0.15 (0.06-0.40)	<0.001	0.34	0.74	2.20 (0.87-5.58)	0.096
4	1.87	0.18	0.09 (0.03-0.26)	<0.001	0.65	0.32	0.49 (0.23-1.02)	0.055
5	7.12	0.52	0.07 (0.03-0.16)	<0.001	3.36	0.90	0.27 (0.12-0.61)	0.002
6	2.51	0.27	0.11 (0.02-0.70)	0.020	0.76	0.18	0.24 (0.09-0.64)	0.004
7	8.70	3.44	0.40 (0.16-0.95)	0.039	2.18	3.39	1.56 (0.56-4.30)	0.392
8	11.80	1.19	0.10 (0.03-0.32)	<0.001	3.13	1.76	0.56 (0.15-2.07)	0.386
9	3.58	1.15	0.32 (0.15-0.70)	0.004	1.53	1.73	1.13 (0.34-3.84)	0.839

*Areas initially selected as intervention and not sprayed during the first year were not included in the analysis of survey 2 and 3.
Odd surveys: April-May, 9 months after the annual IRS round
Even surveys: November-December, 3 months after the annual IRS round

In the intervention valleys, an additional protective effect due to LNs was observed, with a decrease of *Anopheles* density of 79.5% (95% CI: 51.7-91.3), p < 0.001) in the November-December surveys pooled together. The LNs were given to protect the population during the May-June transmission season when the residual activity of the insecticide use for IRS, had

ceased. However, nine months after IRS, a 56.2% reduction in *Anopheles* density associated with LN use narrowly missed statistical significance (95% CI: 0-71.0, p = 0.053).

Malaria sporozoite infection rates (SR) in *Anopheles*. SR was estimated for all specimens, regardless of their physiological status. Before the intervention, the *P. falciparum* SR was 6.2% (27/433) for *An. gambiae* s.l. and 2.2% (13/586) for *An. funestus*. The post-intervention SR (all surveys pooled together) was 1.0% (10/1018) in the intervention valleys and 2.4% (149/6235) in the control valleys (OR: 0.41, 95% CI: 0.22-0.74, p = 0.004). However, the difference was significant only for *An. gambiae* s.l. (OR: 0.39, 95% CI: 0.21-0.75, p = 0.004) and not for the morphological identified *An. funestus* (OR: 0.49, 95% CI: 0.06-3.80, p = 0.493), probably because of the limited number of specimens collected. On the hilltops, no significant difference in SR between control and intervention areas was observed (OR: 0.81, 95% CI: 0.36-1.81, p = 0.605). For the "*An. funestus*-like" species, ELISA tests (459) were negatives for all surveys.

Infective bites by house per month. In the valleys, vector control reduced the infective bites/house/month by 89.6% (95% CI: 62.5-97.1, p = 0.001). The number of infective bites was undetectable in the intervention valleys (Figure 13), except for surveys 5 and 7, where transmission increased but was still lower than in control valleys with DR of 0.08 (95% CI: 0.01-0.74, p = 0.026) and 0.28 (95% CI: 0.06-1.36, p = 0.114) respectively. In the intervention areas (valleys and hill tops), the transmission was reduced by 84.4% (95% CI: 60.1-93.9, p < 0.001) compared to control areas. No effect of LN-use was observed on transmission for the surveys performed three or nine months after the yearly IRS round. Sporozoite rates in fed *Anopheles* used to calculate the number of infective bites were null in both treated houses with nets and without nets in the surveys done three months after the activities.

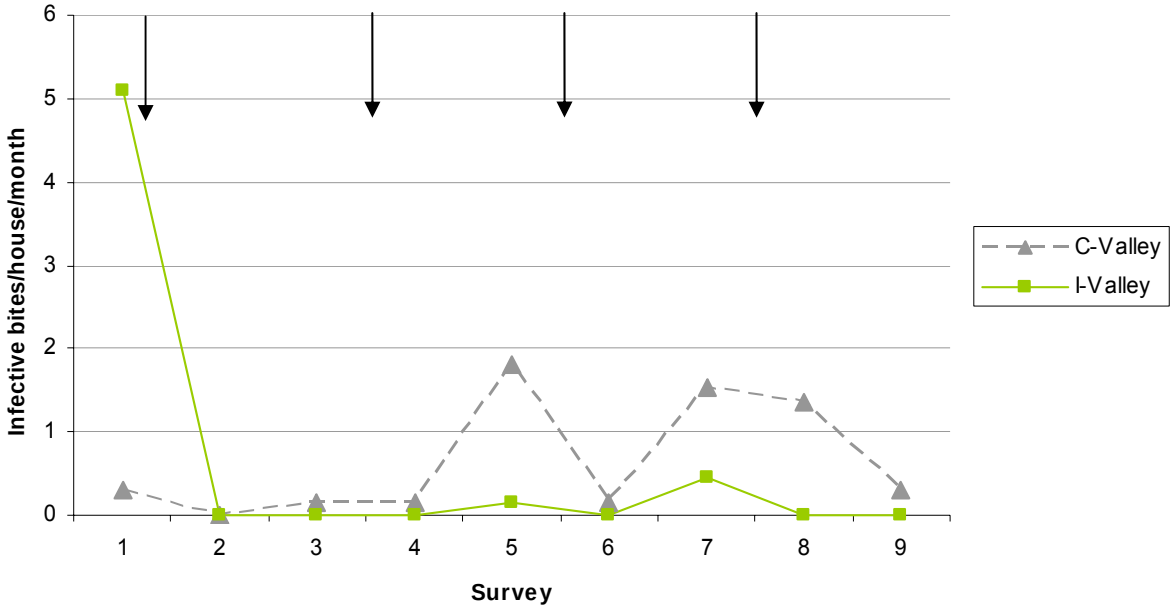

Figure 13: Mean number of infective bites per house by survey in intervention and control valleys. Arrows represent the spraying round. To estimate the transmission only freshly fed females positive with the ELISA test were considered.

Comparison between valleys and hill tops. In survey 1, there was no difference for *Anopheles* density and infective bites/house/month between the valleys and the hilltops in control areas; in the intervention areas 93.1% of the *Anopheles* (95% CI: 78.9-98.1) and 89.6% of the malaria transmission (95% CI: 56.8-98.2) were found in the houses within 700 meters of the valley bottom. These differences were not seen in the intervention areas after the implementation of vector control activities. In control areas, from survey 2 to 9, higher densities of *Anopheles*, sporozoite rates and transmission were found in the valleys compared to the hill tops (Table 10).

Table 10: Comparison of entomological outcomes (indoor resting densities, sporozoite rates and infective bites) between valleys and hill tops of control areas (survey 2 to 9 pooled together).

	Valleys	Hill tops	Ratio* (95% CI)	P value
IRD total *Anopheles* /house	5.2	1.7	3.1 (1.9-5.2)	<0.001
SR total *Anopheles* (No. tested)	2.4% (6235)	1.2% (1898)	2.0 (1.1-3.7)	0.029
IRD fed *Anopheles* /house	1.3	0.5	2.5 (1.3-4.6)	0.005
SR fed *Anopheles* (No. tested)	2.0% (1812)	0.8% (714)	2.4 (0.9-6.1)	0.067
Infective bites/house/month	0.7	0.1	6.0 (2.2-16.8)	<0.001

*Density ratios for Indoor Resting Density (IRD) and infective bites. Odd ratios for Sporozoite Rates (SR).

4.5. Discussion

Vector control based on IRS and insecticide-treated nets are effective tools in preventing malaria in the highlands [26,27]. In order to improve the cost effectiveness of such methods they could be targeted to the malaria high risk areas. Malaria transmission in African Highlands is often focal [28] and breeding sites are usually more common in the valley floors as seen in highlands of Kenya [29], Tanzania [16] and Rwanda-Burundi [13].

In Burundi, from 2002 to 2005, vector control measures combining IRS and LNs were implemented in the highland province of Karuzi. These activities were spatially and timely targeted to enhance the feasibility and lower the cost. One round of IRS per year was organized in June-July before the seasonal increase in transmission. Moreover, only the valley floors, where most *Anopheles* breeding sites are, were treated. The activities were successfully implemented and the high coverage for IRS in the targeted areas has been sustained due to the strong support of the local authorities. The number of LNs retained after distribution among targeted households was rather high for a low income population, no past history of net use and with low mosquito nuisance. However, the life span and fabric integrity of PermaNet® in these poor housing settings was drastically reduced and coverage decreased quickly after the first year, mostly because net were holed by wood sticks and rats and thrown away; some of them were stolen. It appears then that, besides their insecticidal properties, LNs should also be resistant enough to support hard field condition as those occurring in Burundi [30].

This study confirmed that most malaria transmission occurred close to the valley bottoms where rivers, marshes and agricultural activities are. In the intervention areas and before any vector control activity , 93% of *Anopheles* was found near the valley floors, a result consistent with the 98% found by Githeko in the Kenyan highlands [29]. In contrast to what was seen in the Tanzanian mountains [16], the expected protective effect of the treated valleys on the hill tops could not be demonstrated, except for *An. funestus* just after the yearly IRS rounds. This may suggest that the *Anopheles* density in the hilltops, particularly that of *An. gambiae* s.l., may depend also on local and higher breeding places.

Non-treated sentinel houses are commonly used to evaluate a mass effect on the vector population. However, in this study houses in the intervention valleys were selected at random, regardless of their spraying status during the previous IRS round. This method may provide a

more representative picture of the real exposure of the human population. Human landing collections would have been more appropriate to estimate the transmission but this was not feasible because of insecurity. Collection of indoor resting mosquitoes is an alternative when considering the high endophily of malaria vectors in the highlands. The estimation of transmission intensity is then based only on freshly fed females positive for the circumsporozoite antigen by unit of time (month) [24,25].

This spatial targeted intervention drastically reduced the vector populations of *An. gambiae* s.l. and *An. funestus* in the treated valleys compared to the control valleys. By only spraying the valleys, malaria transmission was reduced by 89.6% in the targeted valleys and by 84.4% in the whole intervention areas. Moreover, because the control valleys had a significant lower *Anopheles* density and malaria transmission than intervention area in the baseline survey, the impact of the control measures may be underestimated.

From the fourth year of intervention (year 2005), a lower effect of the control activities on *An. gambiae* s.l. density was observed in the treated areas. This could be attributed to different factors: an overall *Anopheles* increased observed also in the control valleys, a lower quality of the spraying, the use of a different pyrethroid insecticide (alpha-cypermethrin) during the last year and the decreased used and/or efficacy of the LNs. Finally, the repetitive used of IRS could have also selected pyrethroid resistant as recently shown for pre-impregnated plastic sheeting [31]. In Karuzi, an increase in *kdr* allele frequency, involved in pyrethroids resistance in *An. gambiae* s.s. was observed after each spray round and the importance of insecticide resistance would be further investigated. Pyrethroids resistances could hamper malaria control as observed in South Africa [32] and Equatorial Guinea [33].

Usually, IRS or LN's are implemented alone. Recently operational research, to determine the efficacy of combining both interventions areas, has been advocated by the WHO Global Malaria Programme [34]. In the Burundian context, LN-use confers an added value to IRS in reducing the *Anopheles* density in the houses. However, the high coverage achieved with IRS had already decreased the sporozoite rate to undetectable level and no additional reduction on transmission could be observed where LN are used.

Vector control activities of IRS targeting valleys in highland were very effective in reducing *Anopheles* density and malaria transmission. These valleys are responsible for 90% of the

transmission occurring in the area. In sprayed areas, LNs reduced further the *Anopheles* density but not the transmission. Unfortunately, treating the valleys did not confer protection for adjacent hilltops, although the density of mosquitoes was much reduced there. Given limited resources, it appears that such targeted approach in highlands could avoid the spread of epidemic from these foci preventing outbreaks in the whole province.

4.6. Authors' contributions

NP was involved in the study design, conducted the implementation of the operational activities and the data collection, performed statistical analysis and interpretation and drafted the manuscript. WVB was involved in interpretation of the data, helped to draft and revised the paper for intellectual content. TM and MVH were involved in the data analysis and revised the manuscript. PM initiated this programme and revised the manuscript. BD was involved in the operational activities, the data collection and revised the manuscript. MC was responsible for the study design and conception. UDA contributed to the study design and with MC critically reviewed the manuscript for intellectual content. All authors have read and approved the final version of the manuscript.

4.7. Acknowledgements

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Chapter 5. Spatial targeted vector control is able to reduce malaria prevalence in the highlands of Burundi

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5.1. Abstract

In a highland province of Burundi, indoor residual spraying and long lasting insecticidal nets distribution were targeted in the valley, aiming also to protect the population living on the hilltops. The impact on malaria indicators was assessed and the potential additional effect of nets evaluated. After the intervention, and compared with the control valleys, children 1 to 9 years old in the treated valleys had a lower risk of malaria infection (Odd Ratio, OR: 0.55), high parasite density (OR: 0.48), and clinical malaria (OR: 0.57). The impact on malaria prevalence was even higher in infants (OR: 0.14). Using nets did not confer an additional protective effect to spraying. Targeted vector control had a major impact on malaria in the high-risk valleys but not in the less-exposed hilltops. Investment in targeted and regular control measures associated with effective case management should be able to control malaria in the highlands.

5.2. Introduction

Malaria outbreaks have been frequently reported in the African highlands [1-3]. Their occurrence has been attributed to several factors, including climatic anomaly [2,4], land use changes [5], drug resistance [3,6], population migration [7], and breakdown of both the local health system and vector control activities [8]. Different authors have shown that *Anopheles* density, malaria transmission, and corresponding human infections were higher and clustered around the breeding sites [9,10], particularly in the lowly endemic [11] and epidemic-prone areas [12]. Highlands' hilly slopes [13] and cold nights [14] limit upward dispersal of adult mosquitoes from the valleys, accentuating their clustering.

During the last decade, malaria transmission in the Burundian highlands steadily increased and resulted in a major epidemic in 2000. Within a few months (December 2000 to March 2001), 2.9 million malaria cases were reported for a population of 6.7 million. After this epidemic, prevention became an absolute priority for the Burundi Ministry of Health (MoH). However, because of political unrest, most vector control activities (indoor residual spraying and insecticide treated nets) implemented in the lowlands [15,16] and aimed at controlling malaria have been stopped since 1992. Nevertheless, vector control activities were shown to be feasible in the highlands and in the context of a complex emergency situation [17]. Therefore, a four year vector control program was set up in Karuzi, one of the highland provinces most affected by the 2000 malaria epidemic. This was targeted in time and in space,

run between 2002 and 2005, and consisted in an annual round of Indoor Residual Spraying (IRS) only at the bottom of the valleys and one distribution in 2002 of Long Lasting Insecticidal Nets (LNs). Reductions of the vector population and the malaria transmission have already been reported [18]. We present here the impact of these targeted vector control activities on the prevalence of malaria infection.

5.3. Methods

5.3.1. Study area and population

Karuzi is located in the central plateau of Burundi at an altitude of 1,400 to 1,900 m. It is a hilly province with a surface of 1,457 km^2 and an estimated population of 302,062 inhabitants [19]. The annual average temperature is 19°C with the coolest season recorded in June-July and the hottest in September-October. There are two rainy seasons, from September to December and from January to May with an average annual rainfall of 1,160 mm. According to the MoH, malaria in the high plateau is hypo- to meso-endemic and prone to epidemics. An increase of malaria cases is usually observed at the end of the two rainy seasons and recent epidemics occurred after the second one [19]. In 2002, malaria was responsible for approximately 60% of the total out-patient attendances in Karuzi [20]. *Anopheles gambiae* sensu stricto (s.s.) and *Anopheles funestus* are the main vectors [18].

5.3.2. Study design

Vector control activities were described in a previous study [18]. Briefly, four zones were identified in this study: (1) intervention treated valleys (population of 67,187 and area of 264 km^2), (2) corresponding intervention non-treated hilltops (51,161 inhabitants; 201 km^2), (3) un-sprayed control valleys (11,744 inhabitants; 50 km^2), and (4) control hilltops (10,709 inhabitants; 55 km^2). In treated valleys, one annual IRS round was performed in June-July using deltamethrin 5WP (in 2002-2004) or alphacypermethrin 5WP (in 2005) at a concentration of 25 mg active ingredient/m^2. IRS coverage exceeded 90%, except in 2002 (86%). LNs (PermaNet® 1.0) were distributed in 2002, before the first IRS round on the basis of two LNs per sprayed house. Intervention areas correspond to large valleys with many irrigation fields and high population density. The control areas were smaller and were selected to enable the evaluation of the vector control intervention. In both intervention and control areas, people had access to anti-malarial treatment. Nine cross-sectional studies were

performed. The first was carried out before the beginning of the vector control activities to provide baseline data. Then, two yearly surveys were carried out, three and nine months after each annual IRS round. The study was designed to have by survey 80% power to detect 20% difference in malaria prevalence between intervention and control areas, with 95% confidence, assuming a design effect of two and a prevalence of 40% in the control group. A random cluster-sample design was used for every survey. The selection of houses was detailed in a previous paper [18]. Totals of 450 houses in survey 1, 600 houses in survey 2 and 800 houses for survey 3 to 9 were selected. All members of the household were enumerated, and two individuals, one between ages one to nine and one over age nine, were chosen at random, irrespective of any clinical symptoms. When a selected person was not present on the survey day, another appointment was made. Entomological evaluation were carried out in the same houses, and results have been presented elsewhere [18].

Thick and thin blood smears were collected and stained with Giemsa (5% for 20 minutes). Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBC), assuming a total WBC count of 8000/µl. Thin blood films were used to confirm species identification. Quality control was done on 10% of the slides of each survey. When the discrepancy was more than 5% all the slides were re-read. A Rapid Diagnostic Test (RDT: Paracheck-Pf®) was also used. Individuals found to be positive by the RDT were treated according to the MoH guidelines (in 2003 of 30 mg oral quinine per kg body weight over seven days, and from 2004 artesunate (5mg/kg/day) and amodiaquine (10mg/kg/day) for three days). The axillary temperature was measured. A short questionnaire to collect data on age, sex, net use, malaria attacks, and treatment history during the past two months was administered.

To estimate malaria incidence after three IRS rounds, infants 1-11 months old were included in survey 6. In this study, all infants were recruited in the selected houses to reach a number of eights. If the number of infants was not sufficient, additional closest houses were sampled to reach the required number of infants.

5.3.3. Statistical analysis

The following malariometric indices were evaluated: (1) history of malaria-like illness and (2) malaria treatment during the past two months, (3) prevalence of malaria infection (proportion

of positive blood smears for malaria parasites, both sexual and asexual forms) (4) prevalence of high density parasitemiae (proportion of blood smears with more than 5000 parasite/µl among the total number of slide examined), and (5) prevalence of clinical malaria defined as malaria infection and fever (axillary temperature ≥ 37.5°C).

The data were analyzed using the survey logistic regression in Stata 9.2 (Stata Corp., College station, Texas, USA), taking into account the study design. A cluster is a group of four to eight houses, according to surveys and areas. Malaria indicators were analyzed by age group (1-9 and > 9 years) for valleys and hilltops. The malaria indices were first compared between untreated valleys and hilltops (Table 11). Baseline data on population characteristics and malaria indicators in the four zones were summarized with proportions or means (Table 12). The prevalence of infection was analyzed using the following independent variables: survey identification, intervention versus control, and their interaction terms (Table 13). A multivariate regression logistic was used to assess the effect of using a net and living in a sprayed valley on prevalence, clinical malaria, and high density parasitemiae (Table 14). Finally, analyses of malaria infection in 1 to 11 month old infants were done in valleys and control areas with intervention versus control as the main independent variables. This bivariate model included also age as potential confounder.

5.3.4. Ethics

The Ethics Committee of the Institute of Tropical Medicine, Antwerp, approved the study. At the time of the implementation of the program and surveys, the Institutional Ethical Committee was not functional in Burundi. However, the Ministry of Health signed an agreement for the vector control program and the study design and the national malaria control program (LMTC) offered close collaboration. Informed consent was obtained for the individuals or their parents included in the survey. In case of refusal, other houses were selected.

5.4. Results

5.4.1. Malaria in Karuzi

In the untreated zones (all the selected zones except the intervention valley in surveys 2 to 9), children 5-19 years old had the highest prevalence of malaria infection, while individuals over age 50 had the lowest (20.2%). Malaria prevalence was lower in the hilltops than in the valleys but followed a similar trend (Figure 14). *Plasmodium falciparum* was the predominant species (85.2%, 2891/3393), followed by *Plasmodium malariae* (6.7%, 228/3393) and *Plasmodium ovale* (0.5%, 15/3393), with the remaining 7.6% (259/3393) being mixed infections. This distribution was almost constant throughout the surveys.

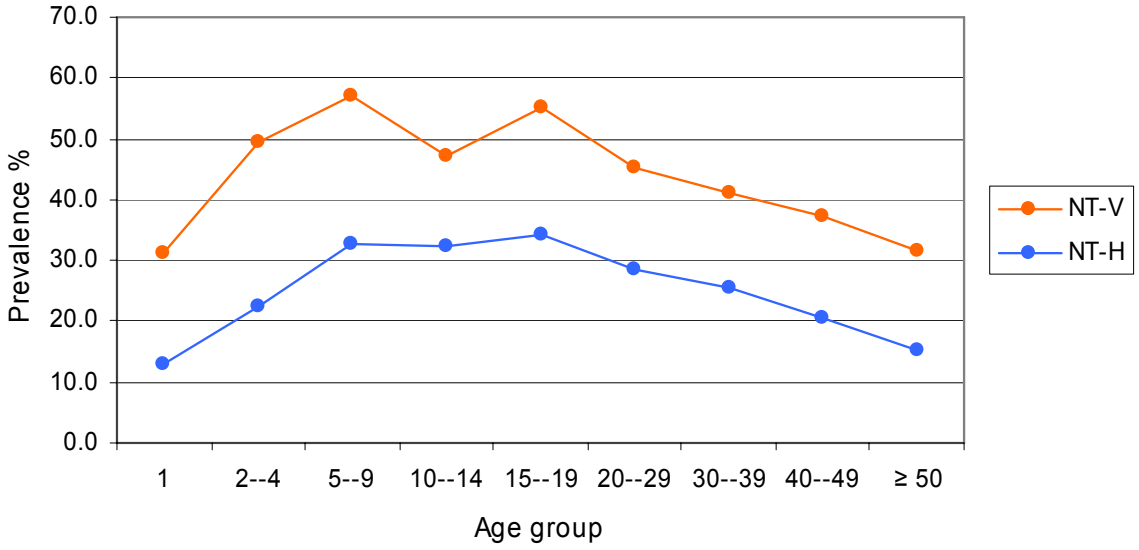

Figure 14: Age-specific malaria prevalence in un-treated valleys (NT-V: valleys of survey 1 and control valleys of surveys 2 to 9), and hilltops (NT-H: intervention and control hilltops of all surveys).

The proportion of individuals to declare a history of malaria-like illness, to have used antimalarial treatment, to be infected with high parasite density, and to have clinical malaria at the time of the survey was significantly higher in the valleys than in the hilltops (Table 11). These differences were seen in both age groups but were the highest in children under age nine.

Table 11: Malaria indices and risk (Odd Ratio) in un-treated valleys (NT-V: valleys of survey 1 and control valleys of surveys 2 to 9) compared with un-treated hilltops (NT-H: intervention and control hilltops of all surveys).

	NT-V	NT-H	OR (95%CI)	P value
Age group ≤ 9 y:				
% history of malaria-like illness (N)	62.3 (1123)	48.3 (2172)	1.8 (1.5-2.2)	< 0.001
% used malaria treatment (N)	27.9 (1122)	22.7 (2172)	1.3 (1.1-1.6)	0.011
% prevalence (N)	52.6 (1072)	27.3 (2090)	3.0 (2.4-3.7)	< 0.001
% high-density parasitemiae (N)	11.0 (1061)	5.0 (2079)	2.4 (1.7-3.2)	< 0.001
% clinical malaria (N)	14.0 (1068)	6.2 (2087)	2.4 (1.8-3.3)	< 0.001
Age group > 9 y:				
% history of malaria-like illness (N)	68.3 (1620)	59.9 (3166)	1.4 (1.2-1.7)	< 0.001
% used malaria treatment (N)	30.5 (1620)	26.3 (3165)	1.2 (1.0-1.5)	0.029
% prevalence (N)	43.1 (1545)	26.0 (3025)	2.2 (1.8-2.6)	< 0.001
% high-density parasitemiae (N)	4.9 (1533)	3.0 (3004)	1.7 (1.2-2.3)	0.003
% clinical malaria (N)	12.6 (1544)	7.4 (3024)	1.8 (1.4-2.3)	< 0.001

All p-value and 95% Confidence Intervals (CI) were determined taking clustering into account

5.4.2. Pre intervention result

Some 776 people were selected for survey 1 (1 to 9 years old: 316, > 9 years old: 458, age missing: 2), and among them 129 (16.6%) were absent. About half of the missing people (52.1%) were male in the 1 to 9 years old and 36.7% for the older age group. For surveys 2 to 9, the number of missing people was much lower, at 6.0% (611/10,127).

In the valleys, the demographic characteristics and the malariometric indices were similar in control and intervention areas (Table 12). In the hilltops, however, some differences were observed in terms of history of malaria-like illness and clinical malaria for the age group > 9 years. The proportion of people sleeping under a bed net was higher in the intervention areas. Most of the hills in the intervention areas were considered to be at high risk during the 2000 epidemic, and these households received LNs in 2001 [17]. The overall parasite prevalence during the first survey was 40.2% (260/647), 17.9% (44/246, count of parasite was not done for 14 slides) had a high parasite density, and 19.7% (51/259, one body temperature was missing) fever.

5.4.3. Post intervention result

When intervention with control valleys were compared, children of age 1 to 9 years had a significantly lower risks of malaria infection (OR: 0.55, 95% Confidence Interval (CI): 0.42-0.72, $p < 0.001$), high density parasitemiae (OR: 0.48, 95%CI: 0.33-0.70, $p < 0.001$), and clinical malaria (OR: 0.57, 95%CI: 0.41-0.81, $p = 0.001$). Furthermore, histories of malaria illness (OR: 0.66, 95%CI: 0.52-0.83, $p < 0.001$) and antimalarial drug use (OR: 0.65, 95%CI: 0.49-0.85, $p = 0.002$) were lower in the intervention valleys compared with the control valleys. The impact of the intervention in the older age group was also significant but less pronounced for all of these outcomes. According to surveys, malaria prevalence was reduced in intervention valleys compared with control valleys by 12% to 64% in the ≤ 9 age group and by 14% to 59% in > 9 age group (Table 13). These differences were significant in children ≤ 9 years old for surveys 3, 5, and 9 and in individuals > 9 years old for surveys 3 to 6 and 9. No difference in malaria prevalence was observed between intervention hilltops and control hilltops (results not shown).

Use of LNs, based on individual declaration of sleeping the previous night under a LN, ranged between 70.2% (217/309) for survey 2 to 18.5% (61/330) for survey 9. LNs use was relatively high until survey 6 (57.7%) and dropped below 36% afterward. When all survey results were combined, the relative impact of IRS and net use varied according to age group, season (nine months after IRS and three months after IRS) and malaria indicators (Table 14). Three months after the intervention, living in a sprayed valley significantly reduced prevalence, clinical malaria and high density parasitemiae compared with houses located in control valley in all age groups, except for clinical malaria, in the > 9 age group. Sleeping under a net did not decrease any of the malaria indicators adjusted for spraying. Nine months after the intervention, when the residual effect of the insecticide used for IRS has ceased, prevalence was still lower in houses located in sprayed valleys for both age groups and also for high parasitemiae in children 1 to 9 years old.

Table 12: Baseline: Demographic characteristics and malaria indices in the intervention and control areas (survey 1).

	Valleys				Hilltops			
	Control		Intervention		Control		Intervention	
Population	11,744		67,187*		10,709		51,163	
Area (km²)	50		264		55		201	
Age group ≤ 9 y:	N = 64		N = 54		N = 57		N = 93	
Mean age in years	6.1	(5.4-6.7)	5.6	(4.8-6.4)	6.1	(5.4-6.8)	5.3	(4.7-5.8)
% of males	46.9%	(34.5-59.2)	59.3%	(44.3-74.2)	49.1%	(34.6-63.7)	55.9%	(43.9-67.9)
% sleeping under a net	3.1%	(0.0-9.1)	14.8%	(0.0-30.8)	0%	-	25.8%	(10.6-41.0)
% history of malaria-like illness	89.1%	(80.8-97.4)	81.5%	(72.2-90.7)	82.5%	(70.4-94.5)	62.4%	(51.2-73.5)
% used malaria treatment	59.4%	(42.3-76.5)	46.3%	(29.5-63.1)	43.9%	(24.6-63.1)	37.6%	(25.6-49.7)
% prevalence	51.6%	(37.9-65.2)	66.7%	(55.8-77.5)	33.3%	(16.9-49.8)	38.7%	(23.5-54.0)
% clinical malaria	9.5%[1]	(1.5-17.6)	13.2%[2]	(1.8-24.6)	10.5%	(0.0-21.0)	12.9%	(3.7-22.1)
% high-density parasitemiae	3.2%[3]	(0.0-7.5)	9.3%	(1.3-17.2)	10.7%[4]	(0.0-21.2)	12.2%[5]	(4.0-20.5)
Age group > 9 y:	N = 79		N = 84		N = 80		N = 136	
Mean age in years	32.9	(28.3-37.6)	36.3	(32.8-39.8)	36.3	(31.9-40.8)	32.8	(30.0-35.5)
% of males	43.0%	(34.2-51.8)	26.2%	(16.1-36.3)	43.8%	(33.9-53.6)	39.7%	(31.0-48.5)
% sleeping under a net	2.5%	(0.0-7.5)	16.7%	(3.5-29.9)	0%	-	21.3%	(8.7-33.9)
% history of malaria-like illness	86.1%	(76.9-95.2)	79.8%	(69.5-90.0)	90.0%	(84.1-95.9)	65.4%	(54.7-76.2)
% used malaria treatment	58.2%	(44.5-72.0)	57.1%	(45.1-69.2)	57.5%	(44.1-70.9)	42.6%	(33.1-52.2)
% prevalence	48.1%	(37.1-59.1)	35.7%	(21.7-49.7)	32.5%	(21.3-43.7)	30.9%	(22.8-39.0)
% clinical malaria	6.3%	(1.0-11.7)	3.6%	(0.0-7.4)	10.0%	(3.1-16.9)	2.9%	(0.0-5.6)
% high-density parasitemiae	6.9%[6]	(1.3-12.4)	3.6%	(0.0-7.4)	7.6%[7]	(1.0-14.2)	4.4%[8]	(1.3-7.5)

Mean and proportion are presented with their 95% Confidence Intervals (95%CI). All 95%CI were determined taking clustering into account
* The population in the intervention valleys was collected during a census done before survey 1; the other numbers were estimated from data given by the administration
N = [1]63, [2]53: Some parasite densities were missing; [3]62, [4]56, [5]90, [6]73, [7]79, and [8]135: Some temperatures were missing

Table 13: Prevalence of malaria infection in children 1-9 years and over 9, observed by surveys and by areas in the valleys. Risk (Odd Ratio) of infection in intervention relative to control valleys.

	≤ 9 years			> 9 years		
	Prevalence (N)	OR (95%CI)	P value	Prevalence (N)	OR (95%CI)	P value
Survey 2						
Control	64.1% (39)	1	0.789	44.1% (59)	1	0.520
Intervention	61.1% (113)	0.88 (0.34-2.28)		38.2% (191)	0.79 (0.38-1.64)	
Survey 3						
Control	64.7% (119)	1	0.040	51.7% (178)	1	0.033
Intervention	44.2% (86)	0.43 (0.19-0.96)		35.8% (123)	0.52 (0.27-0.95)	
Survey 4						
Control	59.2% (130)	1	0.091	45.4% (196)	1	0.048
Intervention	42.5% (134)	0.51 (0.23-1.11)		32.3% (192)	0.57 (0.33-0.99)	
Survey 5						
Control	52.2% (136)	1	0.003	47.6% (185)	1	0.006
Intervention	28.5% (137)	0.36 (0.19-0.71)		26.9% (186)	0.41 (0.21-0.77)	
Survey 6						
Control	38.2% (136)	1	0.105	33.3% (204)	1	0.033
Intervention	26.0% (123)	0.57 (0.29-1.13)		20.8% (192)	0.53 (0.29-0.95)	
Survey 7						
Control	47.7% (128)	1	0.063	40.8% (184)	1	0.218
Intervention	31.7% (123)	0.51 (0.25-1.04)		31.7% (183)	0.67 (0.36-1.26)	
Survey 8						
Control	41.4% (133)	1	0.213	35.1% (185)	1	0.584
Intervention	30.6% (134)	0.63 (0.30-1.31)		31.9% (182)	0.86 (0.51-1.46)	
Survey 9						
Control	57.9% (133)	1	0.049	49.7% (191)	1	0.005
Intervention	39.4% (134)	0.47 (0.22-1.0)		33.5% (200)	0.51 (0.32-0.82)	

All p-value and 95% Confidence Intervals (CI) were determined taking clustering into account
Odd surveys: April-May, 9 months after the annual IRS round
Even surveys: November-December, 3 months after the annual IRS round

Table 14: Impact of Indoor Residual Spraying and sleeping under a net on malaria prevalence, clinical malaria and high density parasitemiae, three and nine months after intervention in the valley. A multivariate logistic regression was used.

	Prevalence		Clinical Malaria		High density parasitemiae	
	OR* (95%CI)	P value	OR* (95%CI)	P value	OR* (95%CI)	P value
3 months after intervention						
1-9 y						
Sleeping under net vs not	1.21 (0.85-1.72)	0.298	1.10 (0.57-2.13)	0.766	1.09 (0.41-2.89)	0.862
House in sprayed valley vs not	0.65 (0.42-0.99)	0.046	0.51 (0.26-0.98)	0.045	0.35 (0.16-0.79)	0.011
> 9 y						
Sleeping under net vs not	1.0 (0.76-1.34)	0.977	0.82 (0.49-1.39)	0.468	1.19 (0.50-2.85)	0.688
House in sprayed valley vs not	0.71 (0.52-0.97)	0.034	0.64 (0.37-1.10)	0.106	0.38 (0.18-0.78)	0.009
9 months after intervention						
1-9 y						
Sleeping under net vs not	0.88 (0.60-1.31)	0.536	0.95 (0.57-1.57)	0.830	0.88 (0.48-1.62)	0.687
House in sprayed valley vs not	0.45 (0.30-0.69)	< 0.001	0.63 (0.39-1.02)	0.059	0.61 (0.38-0.99)	0.045
> 9 y						
Sleeping under net vs not	0.84 (0.61-1.15)	0.271	0.66 (0.41-1.07)	0.091	0.62 (0.31-1.27)	0.191
House in sprayed valley vs not	0.55 (0.40-0.74)	< 0.001	0.74 (0.48-1.13)	0.156	1.03 (0.59-1.80)	0.916

* Odd Ratio (OR) adjusted for IRS and sleeping under a net

The prevalence of malaria infection among the infants examined during survey 6 was 4.6% (33/711), with 24.2% (8/33) having fever and 33.3% (11/33) high density parasitemiae. Malaria prevalence was significantly lower in the intervention valleys than in the control valleys (OR: 0.14, 95%CI: 0.04-0.52, p = 0.005). No difference was observed between intervention hilltops and control hilltops (Table 15). Infants treated for malaria before the survey were significantly less in the intervention valleys (3.7%, 7/189) than in control valleys (9.9%, 16/161; OR: 0.35, 95%CI: 0.13-0.91, p = 0.039).

Table 15: Malaria prevalence and risk of malaria infection in infants 1-11 months old (survey 6).

	n/N	Prevalence	OR* (95%CI)	P value
Valley				
Control	17/161	10.6%	1	0.005
Intervention	3/189	1.6%	0.14 (0.04-0.52)	
Hill top				
Control	6/182	3.9%	1	0.797
Intervention	7/179	3.3%	1.19 (0.30-4.74)	

*OR adjusted for age, all P value and CI were determined taking clustering into account
n/N: Number of positive slide/number examined

5.5. Discussion

In Africa, the spatial distributions of malaria have been extensively studied in low endemic and epidemic-prone areas, and focal vector control activities have been recommended [10,11,14,21]. In Karuzi, vector control activities were based on one distribution of LNs and a yearly round of IRS targeting the valleys, before the main transmission season. This was justified by the observation that 90% of malaria transmission occurred in the valleys [18]. Moreover, the baseline study showed that children are indeed three times more at risk for a malaria infection in the valleys compared with the hilltops.

Although control areas were similar to intervention areas in term of demographic characteristics and malaria indices, bednet use was higher in the intervention areas before the start of the operations. The choice of the intervention areas was done on the basis of the perceived risk for epidemic, i.e., the intervention valleys were considered to be more at risk than those selected as control areas. During the baseline study, malaria transmission was 15 times lower in the control compared to the intervention valleys [18]. Despite this difference and with a drop of infectious bites from 5.1 to less than 0.5 per house per month after the first intervention round [18], we were able to show a significant reduction of all malaria indices in

the intervention valleys compared with control valleys. In a holo-endemic area of Kenya, frequency of exposure to sporozoite-infected mosquitoes was correlated to malaria infection but even more to the high parasitemiae [22,23]. It has been concluded that reduction in high parasite densities would reduce malaria morbidity and mortality [22]. These results are in agreement with our findings, where the most important impact was found on high parasitemiae. The effect was even greater in children ≤ 9 years old, possibly because of their lower immunity. The impact of the intervention tended to decrease in the fourth year and could be linked to a relative increase of *An. gambiae* s.s. density in the intervention valleys, although not as high as in the control areas [18].

The impact of the vector control activities on malaria prevalence was particularly important in infants, with an 86% decrease in risk of malaria infection. This is a strong indication that malaria transmission was drastically reduced by the intervention, as these infants were born after its implementation. Prevalence of malaria infection in the whole population, and more particularly of asymptomatic carriers, was higher than expected for an area defined as low transmission and epidemic-prone. The high prevalence of asymptomatic infections suggests a change to a higher level of endemicity. In such changing situation, malaria prevalence among infants is then a more appropriate indicator of impact of ITN [24] or IRS [25].

During the first two years of the intervention, the first line treatment was sulphadoxine-pyrimethamine (SP), later replaced by an artemisinin-based combination therapy (ACT) because of high SP resistance [26], ACT use should improve cure rates, decrease gametocyte carriage, and may reduce malaria transmission where this is unstable [27,28]. In the control areas of Karuzi, malaria transmission was higher after ACT implementation [18], despite more than 20% of the study population having taken an antimalarial treatment. One reason could be the unreliability of the treatment history by the study population, i.e., antimalarial treatment would not be as frequent as estimated by the survey. However, another reason could be that asymptomatic individuals would maintain a sufficiently large gametocyte reservoir that was able to compensate for the potential reduction of transmissibility in the treated patients. In our study, the observed decrease of malaria indices in the intervention areas can be largely attributed to vector control activities as no influence of ACT on malaria transmission could be detected.

No additional protection by LNs use was observed on any of the malaria indicators three months after the intervention. The absence of impact on malaria morbidity of LNs when implementing IRS was also observed in Eritrea [29], while a mutually additive effect has been reported in Equatorial Guinea [30]. However, in Equatorial Guinea the IRS coverage was only 77% compared to more than 90% obtained in Karuzi. When IRS coverage is high, the additional benefit of treated nets is limited, as shown by the entomological surveys where malaria transmission was already being reduced to an undetectable level after spraying [18].

The upper altitude limit for malaria in the African highlands has risen in past decades, and formerly malaria-free areas have become epidemic-prone [2,4,31]. The spread of the vectors' distribution in time and space expose the local populations to a longer transmission season, which results in an increased endemicity in the highlands [7,32]. In Burundi, at the beginning of the century the central plateaus were declared malaria-free, and then epidemics were reported [5,33,34]. In Karuzi, the high prevalence in children 2 to 9 years old (32.1% to 53.4% in control areas) and the high proportion of asymptomatic carriers show that malaria has become meso-endemic, with a more stable transmission. In epidemic-prone areas emphasis has been put in malaria early warning systems and an early detection systems [35,36], and it was argued that regular vector control measures may be a waste of resources in these areas [37]. However, regarding the spread of malaria in most highland areas, regular vector control activities targeted to the high risk areas could be more cost-effective than less performing emergency interventions often facing delays in mobilization [17].

The IRS activities in Karuzi were stopped at the end of the study, despite ongoing transmission. ACT use alone is unlikely to maintain the reduction in malaria incidence without being associated with preventive measures. In present study, targeted IRS was shown to be very effective to prevent highland malaria and this mainly because of the high coverage. In African highlands, IRS has the advantage to target the places of highest risk [38] (i.e. the valleys). However, effective implementation of IRS relies on highly professional vector control services, good planning and timing of the activities, and strict management and logistics support [39,40]. There is an urgent need to build up this capacity in many places. ITNs especially if they are long lasting have the advantage of being less demanding to implement than IRS and of being able to be targeted at individuals most at risk [41]. As full coverage is essential to impact transmission, both methods can be combined if full coverage with IRS is difficult to achieve or sustain over time. Moreover, the combination of IRS and

ITN could permit better management of insecticide resistance if unrelated insecticides are used [42]. Investment in targeted and regular vector control measures associated with effective case management could have a major impact on malaria morbidity in the African highlands.

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Chapter 6. High *Kdr* increase in the *Anopheles gambiae* population during an intensive vector control intervention in Burundi highlands

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6.1. Abstract

In Burundi, the occurrence of the knock down resistance (*kdr*) mutation in *Anopheles gambiae* sensu lato (s.l.) was determined for six consecutive years in the framework of a vector control programme based on indoor residual spraying and insecticide treated nets. The proportion of *An. gambiae* s.l. carrying the East African LEU-SER *kdr* mutation was 1% before the spraying intervention in 2002; by 2007 it was 86% in the sprayed valleys and 67% in the untreated valleys. A multivariate analysis shows that increased risk of carrying the *kdr* mutation is associated with spraying interventions, location, and time. Bioassays performed in five sites, between 2005 and 2007 showed that *An. funestus* was susceptible to permethrin, deltamethrin and DDT. *An. gambiae* s.l. remained susceptible or tolerant to deltamethrin while DDT and permethrin resistance was confirmed but only once *kdr* allele carriers reached 90%. The cross resistance against DDT and permethrin in Karuzi suggests a possible *kdr* resistance mechanism. Nevertheless, the homozygous resistant genotype alone does not entirely explain the bioassay results, and other mechanisms conferring resistance cannot be ruled out. After exposure to all three insecticides, homozygote individuals for the *kdr* allele dominates among the surviving *An. gambiae* s.l.. This confirms the potential selection pressure of pyrethroids on *kdr* mutation. However, the high occurrence of the *kdr* mutation, observed in sites far from the sprayed areas, suggests a selection pressure other than that exerted by the vector control programme.

6.2. Introduction

Vector control is an essential component of the WHO Global Strategy to roll back malaria. Many studies have shown the efficacy of Indoor Residual Spraying (IRS) and Insecticide Treated Net (ITN) in reducing malaria transmission and prevalence [1-3]. However, these methods, especially ITNs, rely on the use of pyrethroid insecticides and emergence of pyrethroid resistance in vector populations is a major concern for the sustainability of malaria prevention in Africa.

Resistance to pyrethroids in *Anopheles gambiae* sensu lato (s.l.) and to a lesser extent in *An. funestus* has become widespread in Africa [4-9]. Metabolic-based mechanisms and/or a mutation in the sodium channel insecticide target site are responsible for pyrethroid resistance in *An. gambiae* s.l. [10,11]. Knockdown resistance (*kdr*) is caused by a single mutation in the sodium channel, resulting in a leucine to phenylalanine (West Africa mutation) or to serine

(East Africa mutation) change. These two mutations have been held responsible for cross resistance against DDT and pyrethroid insecticides [12,13]. However, the impact of knockdown resistance on the vector control efficacy remains uncertain. In some countries, ITNs can still provide individual protection against *kdr* resistant *Anopheles* populations [14-16] though, more recent studies have shown reduced efficacy when the West African *kdr* mutation frequency is high [17,18]. However, the impact of the East African *kdr* mutation on intervention is unknown.

Resistance in the *Anopheles* species seems to be associated with the agricultural use of insecticides [19,20]. Nevertheless, evidence exists for the selection of *kdr* alleles associated with the massive use of ITNs or impregnated plastic sheeting [8,21]. For insecticide resistance management, it is essential to know where the selective pressure on *Anopheles* comes from.

A targeted vector control intervention combining IRS and ITN was carried out in the highland province of Karuzi (Burundi) between 2002 and 2005 [3] with surveillance continuing for two additional years. The objective of this study is to determine the relative impact of these interventions on the development of insecticide resistance by monitoring the *kdr* mutation in *An.gambiae* s.l. as marker of insecticide pressure, and to link these findings with the insecticide resistance status observed in *An. gambiae* s.l. and *An. funestus* as defined by bioassays carried out by the end of the intervention period. The occurrence of the *kdr* mutation in specimens (homozygote or heterozygote) was preferred to *kdr* allele frequency for statistical analysis purposes.

6.3. Materials and Methods

6.3.1. Intervention programme in Karuzi (2002-2005)

In the central highland Karuzi province (2°54 – 3°23 S, 29°54 – 30°21 E), a four year vector control programme based on IRS and distribution of Long Lasting Insecticidal Nets (LNs), was carried out between 2002 and 2005 [3]. The intervention was targeted to valleys with the highest risk for malaria. IRS was carried out once a year in all human dwellings and cattle sheds of the targeted area (264 km², about 18,000 households) with the residual insecticides deltamethrin 5 Wettable Powder (WP) (from 2002 to 2004) and alpha cypermethrin 5WP (in 2005) at the dose of 25 mg a.i./m². Between 2002 and 2005, respectively 754 kg, 745 kg,

1023 kg and 1080 kg of insecticide were used. Twenty four thousand LNs (Permanet® I) were also distributed during the first year in household selected for spraying.

Between 2002 and 2007, two entomological surveys per year (in April-May and in November-December) using the pyrethrum spray catches were carried out to monitor adult *Anopheles* mosquitoes in treated and untreated areas [3]. One baseline survey was conducted before the intervention (July 2002), 8 surveys were done 3 and 9 months after the annual spray round and 2 surveys were carried out after the end of the intervention. For each survey 25 clusters of 4 to 8 houses were randomly chosen in either treated and in the untreated valleys (range of altitude 1396 to 1717 metres). Specimens of *An. gambiae* s.l. were further analysed for the occurrence of the *kdr* mutation after molecular identification.

6.3.2. WHO insecticide susceptibility bioassays

Between 2005 and 2007, live indoor resting mosquitoes were collected by suction tubes in five different sites to assess the resistance status of the vector species by WHO tube bioassay. Because only few mosquitoes could be collected in the treated province of Karuzi in 2005-2006, three sites were chosen in two communes of the neighbouring province of Gitega, just outside the treated area: commune Mutaho (site 1: 3°09 S, 29°90 E in 2005) and commune Gitega (site 2: 3°38 S, 30°00 E in 2005 and site 3: 3°42 S, 30°02 E in 2006). After the end of the spraying activities, a sufficient number of Anophelines could be collected in two sites in Karuzi, one in a previously treated area (site 4: 3°01 S, 30°16 E) and one in a untreated area (site 5: 3°00 S, 30°19 E) (Figure 15). Individual *Anopheles* were identified using a simplified morphological key adapted from Gillies and Coetzee [22]. Morphologically identified *An. gambiae* s.l. and *An. funestus* were subjected to the WHO standard bioassays [23] with discriminative dosage of DDT (4%), permethrin (0.75%) and deltamethrin (0.05%). The bioassay kit, impregnated and control papers were supplied by Universiti Sains Malaysia, Penang, Malaysia. *Anopheles* mosquitoes were exposed to the insecticide for one hour. Mortality was scored after a 24 hours holding period during which the *Anopheles* had access to 10% sugar solution. Tests with control mortality above 10% were excluded. The bioassay results were divided in three mortality categories according to the WHO criteria [23], i.e. <80% 24 hours post-exposure indicates resistance, 80-97% potential resistance needing confirmation, ≥98% indicates a susceptible population.

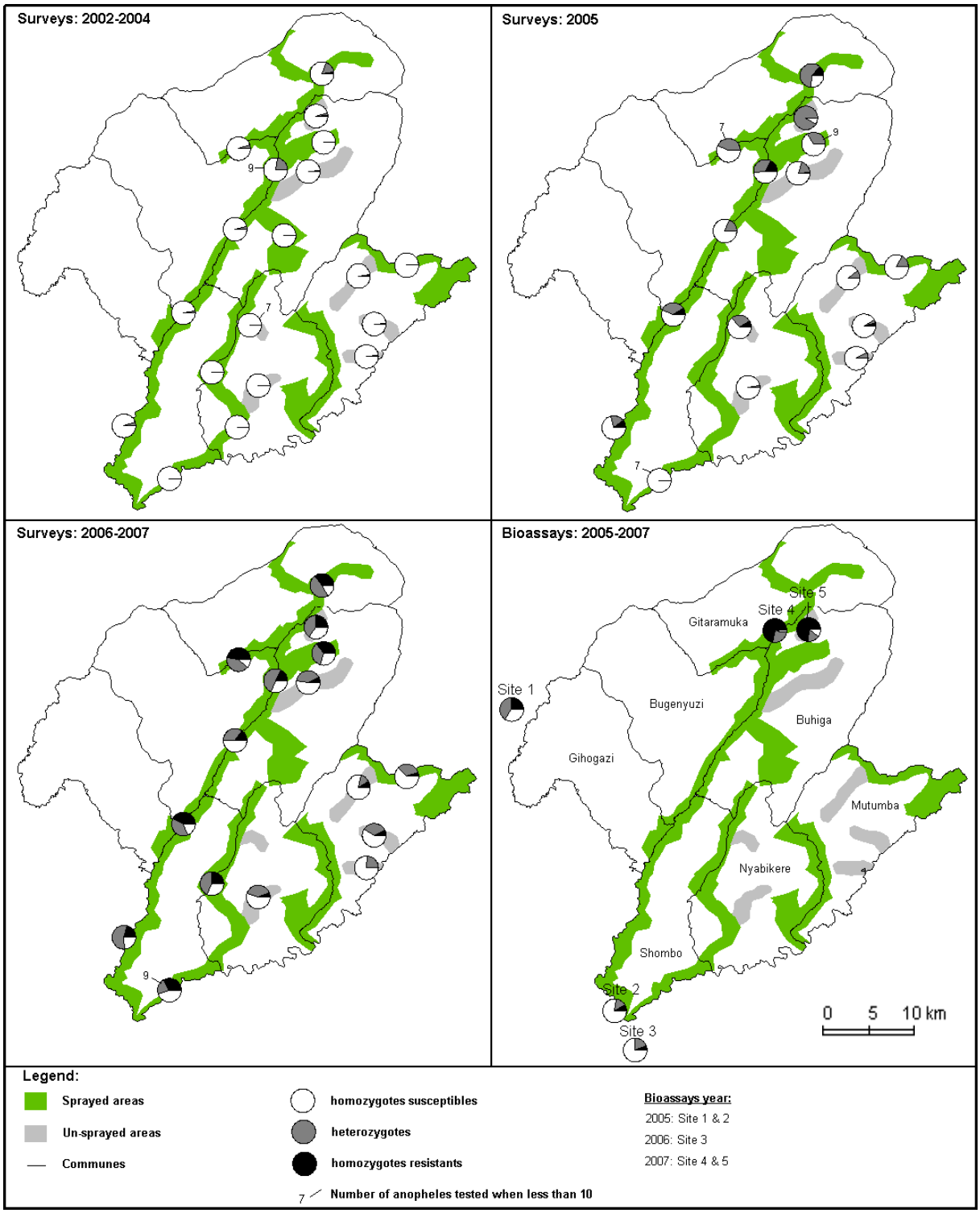

Figure 15: Distribution of the *kdr* genotype of the wild caught A*n. gambiae* s.l. collected in surveys (3 first blocks) and in samples bioassayed (last block).

The pie charts show the relative *kdr* genotypes proportion. Homozygotes for the *kdr* mutation (RR) are in black, heterozygotes (RS) in grey and susceptible homozygotes (SS) in white. Results were summed to reach at least ten *Anopheles* tested (when fewer were tested, the numbers are displayed on the map).

6.3.3. Molecular identification and knock down resistance detection

An. gambiae s.l. and *An. funestus* mosquitoes were morphologically identified. Samples of *An. gambiae* complex collected during the surveys and for the bioassays were tested using a Polymerase Chain Reaction (PCR) adapted from Scott *et al.* [24] to distinguish the different member species. Two hundred and twenty two *An. funestus* from the bioassays were identified following the protocol of Garros *et al.* [25] to assess the reliability of the morphological identification.

The presence of the East African *kdr* mutation in *An. gambiae* s.s. and *An. arabiensis* on specimens collected during the entomological surveys was assessed using an adapted version of the allele specific PCR developed by Martinez-Torres *et al.* [13] and described in Verhaegen *et al.* [26]. A Fluorescence Resonance Energy Transfer / Melt Curve Analysis assay (FRET/MCA) [26] was used to detect the East and West African *kdr* mutation in all the *An. gambiae* s.l. that survived the bioassay tests and in a fraction (1/3) of the dead mosquitoes. The FRET/MCA technique was also used for quality control of the allele specific PCR on a sample of the survey specimens (n=264) and to check for the possible occurrence of the West African mutation (n=1082, combination of surveys and bioassays). Homozygote and heterozygote *An. gambiae* s.l. for the *kdr* mutation are presented as RR and RS and absence of *kdr* mutation by SS.

6.3.4. Statistical analysis

The proportion of *An. gambiae* s.l. collected in the spray-catch surveys that had either the homozygous resistant (RR) or heterozygous (RS) *kdr* genotype was analysed in a robust multivariate logistic regression in Stata 9 (Stata-Corporation, USA, version 9.2). Communes, year of collection (2 surveys a year) and vector control activities (intervention versus control valleys) were used as discrete explanatory variables. Clusters were defined as primary sampling units and sampling weights were used to correct for the proportion of the mosquitoes tested from each house. Genotype frequencies between dead and alive mosquitoes in bioassays were compared using the software Genepop (version 3.4). The global estimation of the *kdr* occurrence in the *An. gambiae* s.l. population was obtained from a weighted average of the proportions of the *An. gambiae* s.l. dead and alive carrying the *kdr* allele.

6.4. Results

6.4.1. Occurrence of the *kdr* mutation during the intervention and post intervention periods.

A total of 9,473 *An. gambiae* s.l. females were caught during the eleven surveys, with only 0.4% *An. arabiensis*. None of the *An. arabiensis* (n=37) were carrier of the East or West African *kdr* mutation. Using the FRET/MCA, the West African *kdr* mutation was not identified in the screened *An. gambiae* s.s.. The quality control, done with the FRET/MCA, showed for the East African *kdr* mutation only one discrepancy (n=264) with the result of allele specific PCR. Before the start of the intervention, the East African *kdr* allele was detected in 1 % (4/404) of the *An. gambiae* s.l. and only in heterozygous genotypes. Between 2002 and 2004 and in the intervention valleys, the occurrence of the *kdr* mutation increased three months after the spray round and decreased six months later (Figure 16). However, form 2005 onwards, *kdr* carriers steadily increased both in the treated and the control valleys

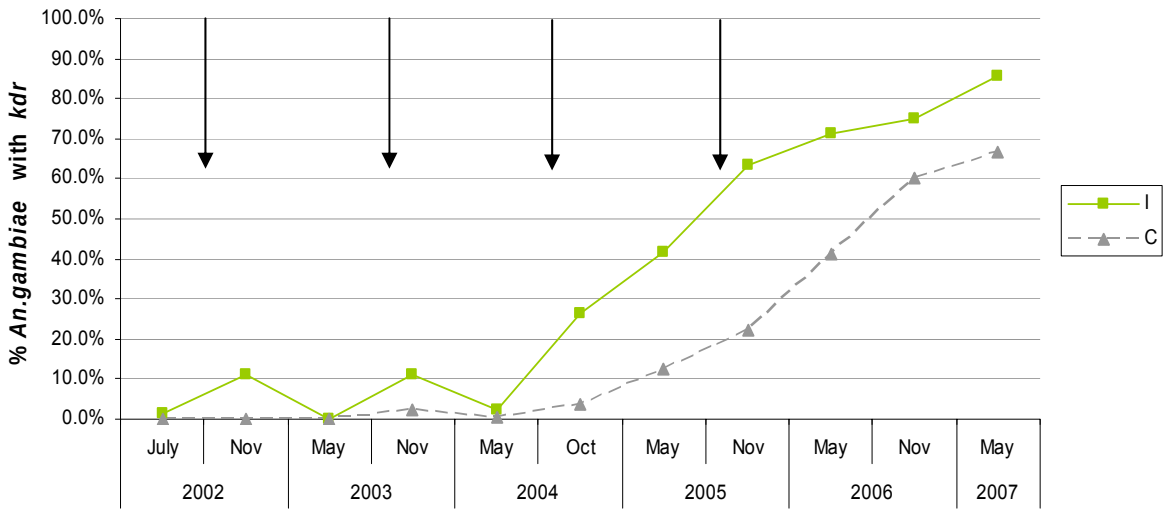

Figure 16: Occurrence of the East African *kdr* mutation in *An. gambiae* s.l. in intervention (I) and control (C) valleys between 2002 and 2007.

Arrows represent the spraying times. The global estimation of the *kdr* occurrence in the *An. gambiae* s.l. population was obtained from a weighted average of the proportion of mosquitoes tested from each house.

The spatio-temporal distribution of the *kdr* genotypes is shown in Figure 15 (first 3 maps). Between 2002 and 2004, the SS genotype in *An. gambiae* s.l. was predominant in the entire province. Homozygote for the *kdr* mutation (RR) appeared by the end of 2004. Between 2006 and 2007, RR and RS genotypes became predominant in most districts. Location (communes), time (year), and spraying were found to be positively associated by multivariate analysis with the proportion of the *An. gambiae* s.l carrying the *kdr* mutation (Table 16). Treated valleys had a 2.7 (95% CI: 1.4 – 5.2) greater risk of having *An. gambiae* s.s. carrying the *kdr* mutation. When compared to the year 2002, this risk increased significantly after 2004, and reached the highest value in 2007 (OR: 168.6, 95%CI: 70.2 – 405.1).

Table 16: Multivariate analysis showing the risk (OR) to have *Anopheles gambiae* s.l. carrying the *kdr* allele (either in the heterozygous or homozygous form) in relation to vector control activities, location and time.

			Multivariate analysis		
	n	% *kdr* mutation	OR	CI 95%	P value
Valleys					0.003
Untreated	1233	13.3%	1.0		
Treated	566	53.3%	2.7	1.4-5.2	
Net used					0.357
0	1664	19.0%	1.0		
≤1	135	35.0%	0.7	0.4-1.4	
Communes					<0.001
Mutumba	554	5.7%	1.0		
Shombo	428	20.5%	2.4	1.1-5.4	
Buhiga	415	23.1%	2.7	1.3 -5.8	
Nyabikere	169	35.0%	3.3	1.8-5.9	
Bugenyuzi	93	52.8%	3.7	1.8-7.6	
Gitaramuka	140	70.5%	6.3	2.5-15.8	
Years					<0.001
2002	395	1.1%	1.0		
2003	220	1.0%	1.2	0.3-4.2	
2004	377	1.8%	2.3	0.9-5.7	
2005	411	23.5%	22.5	9.6-53.1	
2006	268	52.8%	62.6	28.9-135.8	
2007	128	82.6%	168.6	70.2-405.1	

6.4.2. Resistance status of *An. gambiae* s.l. and *An. funestus* as defined by bioassays

Morphological identification was good as only 2 specimens of the 711 molecular tested *An. gambiae* s.l and 4 of the 222 tested *An. funestus* (1.8%) were misclassified. *An. arabiensis* represented only 1% (7/709) of the *An. gambiae* complex. The responses of *An. funestus* and *An. gambiae* s.l. to 4% DDT, 0.75% permethrin, and 0.05% deltamethrin are given in Table 17. *An. funestus* was almost susceptible to these insecticides (mortality >95%). *An. gambiae* s.l. was susceptible to deltamethrin in all sites, except in site 4 (Karuzi) where possible resistance can occur. Outside the province, only suspected permethrine resistance (mortality >80%) was observed for *An. gambiae* s.l., but in site 4 located in Karuzi a high level of permethrin resistance was detected (mortality of 57%). Similarly high DDT resistance was found in Karuzi (site 4 and 5), and possible resistance in site 3.

Table 17: WHO susceptibility test results on *An.funestus* and *An.gambiae* s.l. reporting the % mortality 24 hours post exposure in different sites

			DDT 4%		Permethrin 0.75%		Deltamethrin 0.05%	
Species	Locations	Sites[1] (Years)	n	% mortality (no.)	n	% mortality (no.)	n	% mortality (no.)
An. funestus	Mutaho	N° 1 (2005)	99	98% (97)	94	99% (93)	104	100% (104)
	Gitega	N° 2 (2005)	-	-	60	100% (60)	-	-
	Gitega	N° 3 (2006)	92	98% (90)	86	97% (83)	-	-
	Karuzi	N° 4 (2007)	96	97% (93)	94	99% (93)	86	100% (86)
	Karuzi	N° 5 (2007)	81	95% (77)	83	100% (83)	101	100% (101)
An. gambiae	Mutaho	N° 1 (2005)	102	98% (100)	153	87% (133)	80	99% (79)
	Gitega	N° 2 (2005)	31	100% (31)	83	93 % (77)	-	-
	Gitega	N° 3 (2006)	101	96% (99)	107	84% (90)	101	100% (101)
	Karuzi	N° 4 (2007)	98	58% (57)	189	57% (108)	177	94% (167)
	Karuzi	N° 5 (2007)	19	79% (15)	-	-	20	100% (20)

[1]Site location can be found on figure 2 part bioassays, n= sample size

In sites 1 to 5, the *kdr* mutation was present in 64.9%, 22.6%, 25.2%, 97.6% and 89.6% respectively of the *An. gambiae* s.l, specimens. The RR genotype was largely predominant in site 4 and 5 (Figure 15, block 4). No *kdr* mutations were observed in *An. arabiensis* (n=6). The frequency of *kdr* genotype in dead and alive mosquitoes 24 hours post exposure and by insecticide is presented in Figure 17. The proportion of *kdr* genotypes were significantly different between survivors and non survivors and this for all insecticides tested. In mosquitoes that survived the frequency of RR genotype was 75%, 93% and 100% after exposure to permethrin, DDT and deltamethrin, respectively. Furthermore, SS genotypes were mostly found in dead *Anopheles*, though RR genotype occurred also in dead *An. gambiae* s.l..

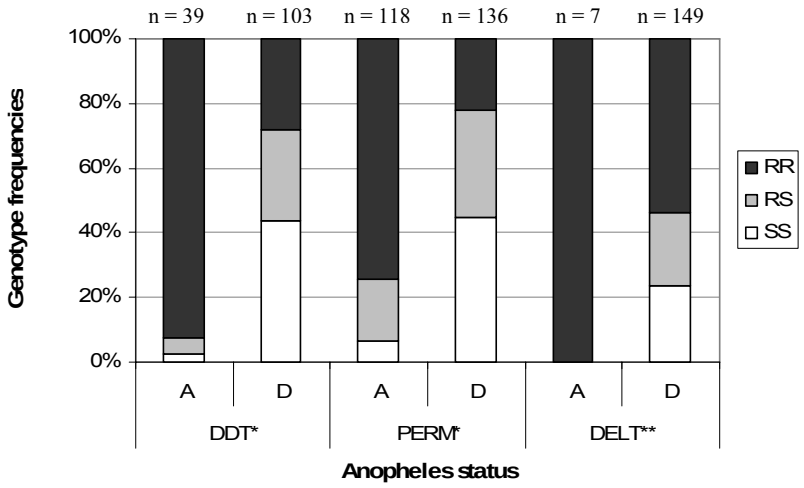

Figure 17: *Kdr* genotypes frequencies found in live (A) and dead (D) *An.gambiae* s.l. 24 hours after exposure to discriminative dose of insecticides.
The p-value (*p < 0.001, **p = 0.019) indicates significance in the difference of genotype between dead and alive mosquitoes.

6.5. Discussion

Selection of the knockdown resistance mutation in West Africa has been mainly attributed to the intensive use of DDT and pyrethroids in agriculture as well as to the DDT-based vector control campaigns undertaken in the 1950's [27,28].

In Karuzi and before starting vector control activities, the occurrence of the East African *kdr* mutation was 1% in *An. gambiae* s.l.. Between 2002 and 2004, it increased temporarily three months after each spray round and dropped down to baseline values nine months later. This

phenomenon lasted only for two years. Indeed, from the second half of 2004 onwards, a steady increase of the *kdr* mutation carriers was observed, both in the treated and untreated valleys, though higher in the former ones, and reaching 60% in less than three years. It has been argued that IRS exerts a much stronger selective pressure than ITNs for insecticide resistance because resistant fed females would fly away from treated surfaces of sprayed houses while unfed females searching for a blood meal, would have repeated and longer contacts on ITNs and would be killed as readily as susceptible ones [21,29]. Indeed, in our study the occurrence of the *kdr* mutation was not significantly different in houses having at least one ITN than in those with no ITN. The high percentage of resistant homozygous *An. gambiae* s.l alive after exposure to deltamethrin in the bioassays could indicate the strong selective pressure exerted by the IRS. However, it should be reminded that this conclusion is based only on seven survivors *An. gambiae* s.l..

The spread of resistance genes in a treated region will depend on the initial *kdr* frequency, the degree of dominance of *kdr* allele and the importance of migration relative to the selection pressure [30]. The steady increase of the prevalence of the *kdr* mutation observed both in the treated and untreated valleys may be explained by several factors. The *kdr* mutation may have migrated from treated to untreated valleys, explaining the parallel increase in these areas, though this occurred only after the third IRS round. Conversely, the higher occurrence of *kdr* in *An. gambiae* specimens observed in the treated valleys compared to the untreated valleys, despite the fact that they are interspersed, could suggest a restricted migration of *An. gambiae* s.l. preventing a massive influx of susceptible individuals from the untreated areas. Once the *kdr* allele frequency reaches a certain threshold and this combined with a drastic decrease of vector densities by IRS, an exponential increase of the resistant forms can be observed in a short period of time. May *et al* [30] stated that when the dominance of the resistant allele is low (<0.5), which is the cases for the *kdr* allele (0.41 reported in *Culex pipiens* and *Aedes aegypti* exposed to permethrin) [31], the system settles to a state of high *kdr* frequency if migration is small and selection overcome gene flow. If migration is restricted, the selection pressure in the untreated valleys may be caused by a selection pressure different from the one induced by IRS. Indeed, the high occurrence of *kdr* mutation observed in the neighbouring province (up to 69% in Mutaho site 1 in 2005), far from the treated valleys, suggests that selection of the resistant form has been caused by pyrethroids used for other purposes than the IRS, though it is difficult to identify the specific activity with the present study. The only record of massive insecticide use in this area was in 1956 when all the houses of Burundi up

to 2000 metres were treated with DDT. Since no specific vector control activities were carried out in the highlands. Domestic use of insecticide (mosquito coils, aerosols) was rare or non-existing during the study period. In this region, the only official record of insecticide use is the treatment of coffee stalks with lambda-cyhalothrin while the population, being extremely poor, did not have access to insecticide for treating subsistence crops. Moreover, though the number of coffee stalks has always be more important in the northern part of the province this does not correlates with the occurrence of the *kdr* mutation.

In bioassays, the homozygous resistant genotype dominates among survivors, but does not explain entirely the bioassay results. Also for other mosquito species, no clear correlation was described between the presence of a *kdr* mutation and the resistance phenotype [32,33]. In *Culex quinquefasciatus*, a high correlation was only found between *kdr* allelic expression and levels of insecticide resistance via transcriptional regulation [32]. However, in our study it cannot be ruled out that in addition to the *kdr*, metabolic based resistance mechanisms may also be involved. Bioassays results in Mutaho and Gitega show a possible association of the *kdr* mutation with permethrin resistance but not with DDT resistance and in Karuzi high level of *kdr* mutation coincides with a similar level of resistance for DDT and permethrin. This is in contradiction with the findings of Ranson *et al.* (2000) who found that the East African *kdr* mutation conferred DDT resistance, and to a lesser extent permethrin resistance.

IRS efficacy changed during the study period. Whereas the *Anopheles* density during the first three years of the spraying campaign was reduced to less than 0.5/house, it was higher than 1/house in the three surveys done in 2005 and 2006, though still significantly lower than the untreated valleys [3]. The West African *kdr* mutation has been held responsible for the decreased efficacy of IRS against *An. gambiae* in Equatorial Guinea [18] and Benin [34] and the East African *kdr* mutation could have a similar effect in Burundi. It is therefore remarkable that in Karuzi, after intensive use of type II (α-cyano-) synthetic pyrethroids in the IRS campaign during five years, mosquitoes were still extremely susceptible to deltamethrin as shown by the bioassays two years after stopping the intervention and this despite the presence of the East African *kdr* mutation in 80% of the *An. gambiae* s.l.. Therefore, the use of the East African *kdr* mutation as a marker of pyrethroid resistance must be employed with caution. Probably the lower-than-expected efficacy observed have several, non mutually exclusive explanations, i.e. a general increase of the A*nopheles* population due to meteorological factors, the use of a different pyrethroid insecticide (alpha-cypermethrin) in

2005 and/or the decrease of the LLINs coverage, LLINs use having an additional impact on *Anopheles* reduction in sprayed houses [3]. Because this increase was observed in all intervention areas a lower quality of spraying was excluded.

In Burundi, the national malaria prevention programme is based on LNs distribution to children and pregnant women and on IRS (pyrethroids) in the high risk areas. Assessing and monitoring insecticide resistance in the malaria vectors should be a priority for the sustainability of the current malaria preventive activities in Burundi. Moreover, resistance management strategies should be implemented to delay emergence or expansion of insecticide resistance. Pyrethroids insecticide should be preserved only for ITN's, while non-pyrethroid insecticides as carbamates or organophosphates should be used for IRS. Rotation, mixtures or mosaic of different classes of insecticide that have different target sites should be also further evaluated for resistance management in the future.

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Chapter 7. Malaria risks in the highlands: the case of Burundi

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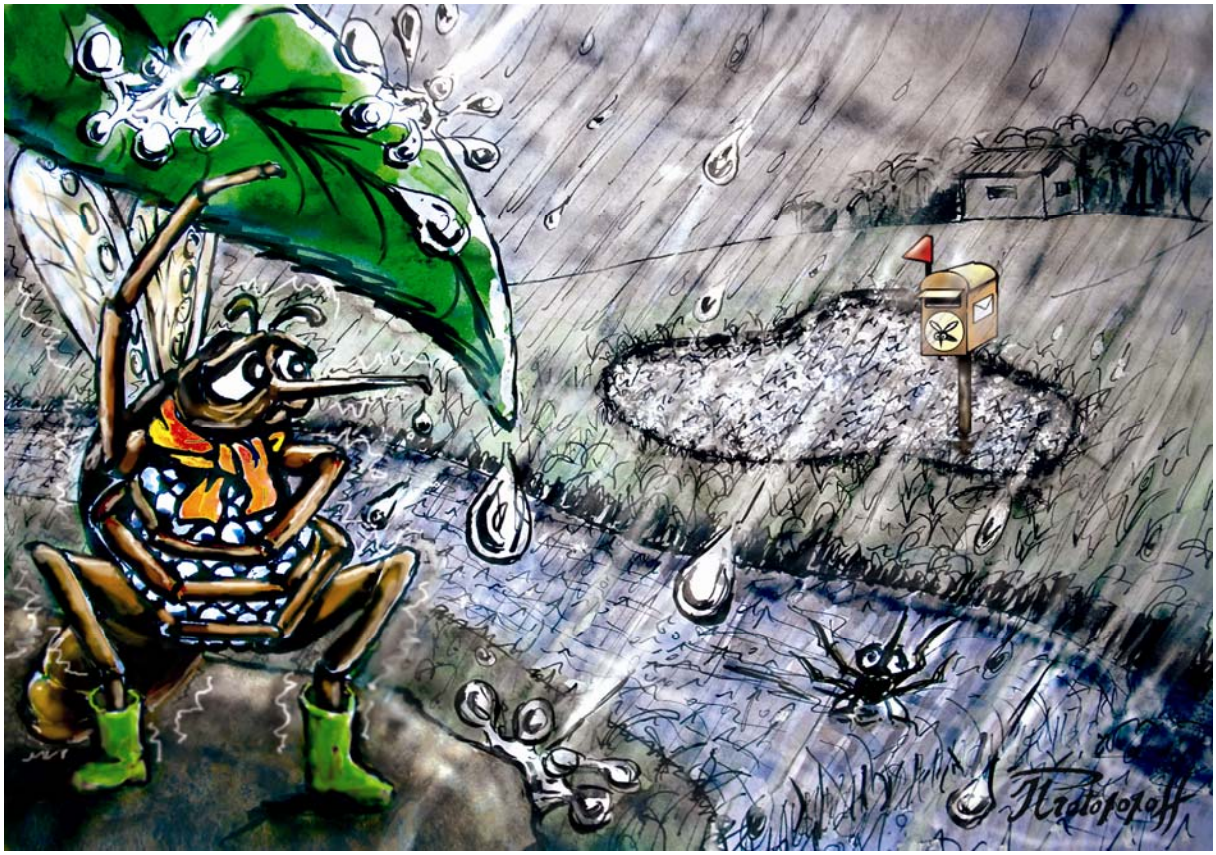
In preparation



7.1. Abstract

Malaria is re-emerging in most of the African highlands. Understanding the factors driving this change can improve the implementation of efficient malaria control strategies. A conceptual model of potential risk factors on the basis of the available literature was built and the relative impact of several parameters collected in Burundi on vector density and malaria prevalence was assessed through the classification and regression trees method. In Burundi, lower rainfall, absence of vector control measures (indoor residual spraying and insecticide treated nets), higher minimum temperature and houses near breeding sites were associated by order of importance to higher *Anopheles* density. The best predictors for high malaria prevalence were more than one resting *Anopheles* mosquito per house and, to a lesser extent, poor housing conditions and age <39 years. In the highlands of Burundi, the follow up of the residual *Anopheles* densities when rainfall is low could be helpful in predicting or in the early detection of epidemics. The CART approach should be consider for future work as it can deal with large number of variables and can explore their relative importance.

7.2. Introduction

Since recently, a resurgence of malaria in the African highlands has been reported [1,2]. Understanding factors fuelling these changes are essential for containing epidemics. Several risk factors have been already identified, including climate [3], environmental changes such as deforestation [4], irrigation [5,6] and swamp drainage for cultivation [7,8], social and economic pressure as population growth [9,10], health system access and quality [2], antimalarial drugs resistance [11,12] and vector control efficiency [13]. However, only a few of them are usually assessed in each specific study, giving only a partial picture of the malaria problem in different settings.

In this paper, an attempt is made to build a conceptual model representing the potential malaria risk factors mentioned in the literature and possibly influencing the malaria situation in the highlands. Available parameters from a database from Burundi are analysed using a statistical method based on classification and regression trees in order to evaluate the importance of these variables on the occurrence of malaria in the highland province of Karuzi.

7.3. Material and methods

7.3.1. Conceptual model of malaria risks based on literature review

An assessment of the different malaria risk factors in the highlands was done based on a literature review and was further used to build a conceptual model. The main source of information was the peer-reviewed scientific papers obtained through Pubmed with the keywords malaria and highland. Each paper describing malaria potential risk factors were retained. The reported risk factors were further linked either for their impact on vectors or on malaria prevalence. Some of them could be inter-related or overlap each other.

7.3.2. The Burundi database

In the central highland province of Karuzi (2°54 – 3°23 S, 29°54 – 30°21 E), a four year vector control programme was based on one annual round of Indoor Residual Spraying (IRS) carried out between 2002 and 2005 and just one distribution of Long Lasting Insecticidal Nets (LNs) in 2002 [14]. The intervention was targeted to valleys presenting the highest risk for malaria. Every year from 2002 to 2007, two cross sectional surveys were carried out in May and in November (11 in total). The detailed sampling process has been described elsewhere [14]. Briefly, for each survey, houses were re-sampled in treated and un-treated areas, 450 houses in the first survey, 600 houses in the second survey and 800 houses in the next surveys. In each selected house, indoor resting *Anopheles* mosquitoes were collected by spray pyrethrum catches and a blood slide was made on two randomly selected persons (≤9 and >9 years old) [14]. Information on location, housing construction (house size, open eaves, type of wall and roof), livestock, separate kitchen, vector control activities (net use and spraying), past treatment against malaria, sex and age was also collected. Altitude and distance to the marsh were registered with a hand held positioning system (GPS 76, Garmin®). Mean monthly minimum and maximum temperatures and monthly rainfalls, of the Karuzi meteorological station, were obtained from the Institute of Geography of Burundi (IGEBU).

7.3.3. Statistical methodology

To understand the interplay between the available variables in the dataset and the density of anopheline species and the probability of positive slides, Classification and Regression Trees (CART) were used. CART is a non linear and non parametric technique that can select among a large number of variables those and their interactions that are most important in determining the dependant variables to be explained [15]. A regression tree attempts to predict the values

of a continuous variable (i.e. number of *Anopheles*) from one or more continuous and/or categorical predictor variables, while a classification tree try to predict values of a categorical dependent variable (i.e. positive slide). The construction of the trees involves a first node containing all the observations. For the first split, CART finds the best explanatory variable to divide the node into two sub-nodes. The splitting is repeated along the sub-nodes until a terminal node is reached. For both trees (classification and regression), the one standard deviation rule was applied to select the best tree (the smallest tree within 1 standard error of the minimum error tree). The minimum terminal node size of 500 samples was selected. CART also ranks the variables by their overall discriminatory power. The score of a particular variable is determined by the sum across all nodes in the trees of the difference at each node between the discriminative power of this variable and the discriminative power of the best splitter [16].

7.4. Results

7.4.1. Conceptual model for malaria risk in the highlands

Due to the instability of transmission and the low immune status, all the age groups are normally at risk in the highlands [2,17]. Therefore, small variations in environmental or human related factors could increase transmission leading to dramatic consequences. Different non-exclusive factors can interact to drive these changes. First, vectors and parasites are strongly dependant of the temperature and a small rise either due to seasonal variability [18], local microclimatic changes due to land use [19] or to global warming [20,21] could increase malaria distribution. Unusual heavy rainfalls have a direct impact on breeding sites availability and vector densities. Indeed, in Ethiopia [22] and Uganda [23,24], extreme rainfall was associated with malaria epidemics whereas in Tanzania malaria decreased [25] as breeding sites were probably flushed away. Secondly, land use changes favour the proliferation of vector by increasing the local temperature **[4]** and/or by creating new habitats for effective vectors such as *Anopheles gambiae* and *Anopheles funestus* [8,26-28]. However, environmental management could also reduce the availability of these breeding sites [29-31]. In different highland settings, *Anopheles* is highly clustered and its density is negatively correlated with the increasing distance between breeding sites and houses [14,32,33]. Vector control, such as Indoor Residual Spraying (IRS) and Insecticide Treated Net (ITN) with a certain coverage, has a direct impact on the transmission by reducing *Anopheles* densities and sporozoite rates [14,34]. However, the emergence of insecticide resistance may decrease the

effectiveness of these methods [35,36]. Socio-economic status has an impact on the type of housing and a higher number of mosquitoes were found in poorly constructed houses [37,38]. In addition, other factors, such as population migrations [39], keeping livestock inside the house [38,40], collapsing health systems [2] or health status (malnutrition and HIV) [41,42] have also created ideal conditions for the increased burden of malaria. Intermittent preventive treatment is able to reduce morbidity and mortality in pregnant women and infants [43,44]. Finally, in several countries, resurgence of malaria has been largely attributed to the emergence and spread of drug-resistant parasites [45-47], whereas implementation of effective treatment such as artemisin-based combination therapy (ACT) has resulted in a decrease in transmission in low endemic areas [48,49].

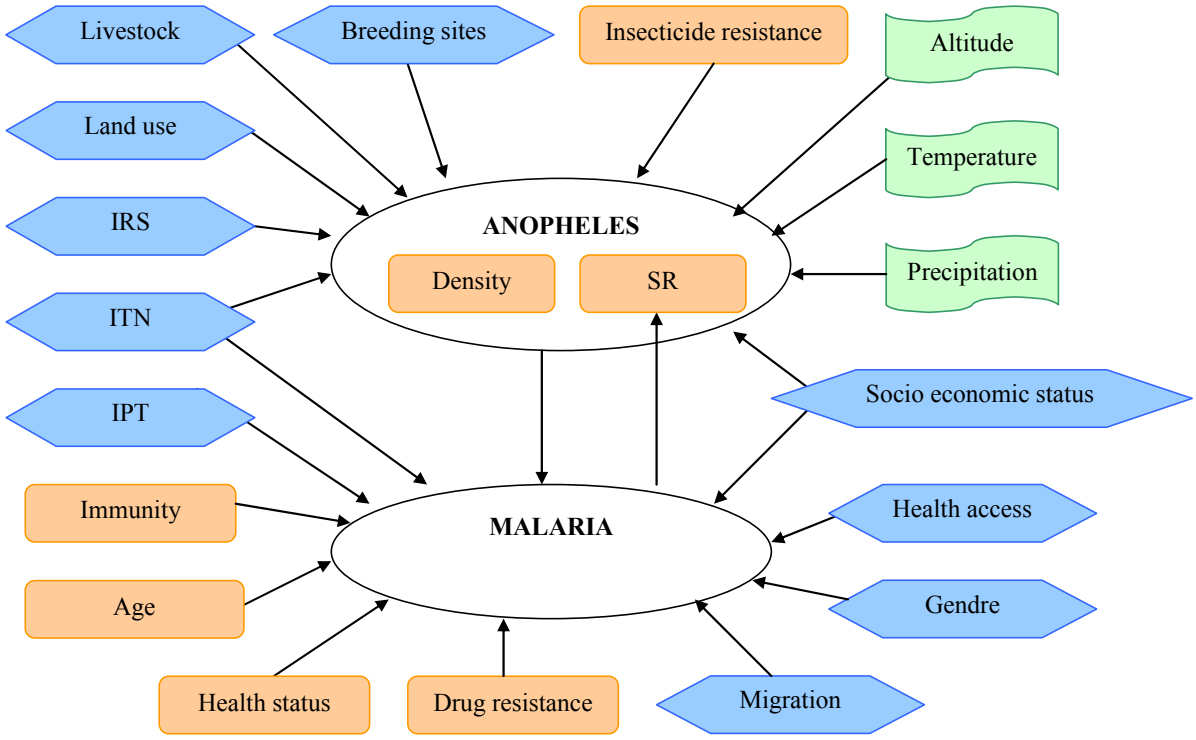


Figure 18: Conceptual model of important risk factors affecting malaria prevalence in the African highlands based on literature review.
Factors are regrouped in 3 main classes (environmental factors: green label, biological factors: orange label and human related factors: blue label). ITN = Insecticide Treated Nets, IRS = Indoor Residual Spraying, IPT = Intermittent Preventive Treatment, SR = Sporozoite Rates

The conceptual model of potential factors influencing either *Anopheles* (density or sporozoite rates) or malaria prevalence in the highland based on this review is presented in Figure 18. They are regrouped in three classes [1,2], 1/ environmental factors: altitude and climate

(temperature and precipitation), 2/ biological factors: related to *Anopheles* (insecticide resistance and density), related to parasite (drug resistance and sporozoite rate) and related to the human host (immunity, age and health status) and 3/ human related factors: socio-economic status, health access, migration, gender, control activities (IRS, ITN, IPT), land use changes, and availability of breeding sites.

7.4.2. Case of Burundi

Based on the conceptual model, available variables from the Burundi database chosen among the 3 classes presented in figure 18 (environmental, biological and human related) were used in the CART analysis to explain either the *Anopheles* density or the probability of having a positive slide. For *Anopheles* density the variables chosen are monthly rainfall and average monthly minimum and maximum temperatures (during the month of the survey and 1 and 2 months before the survey), housing conditions, vector control activities, and environmental parameters. For malaria infection these factors are *Anopheles* density, individual characteristics, housing condition, past treatment, and survey (Table 18). Housing condition was divided in 4 categories. This variable was constructed taking into account: the house size (floor area size; <25 m^2, 25-50 m^2, >50m^2), type of wall (thatch, mud, and bricks), type of roof (thatch, mix or plastic sheeting, iron sheet, and tiles), open eaves (yes, no) and separate kitchen (yes, no). A typical poorly constructed house (category 1) is a small house (<25 m²) with thatch walls and roof, open eaves, and no independent kitchen. The best constructed house (category 4) is a larger house (>50 m²) with walls made of bricks and roof with iron sheet or tile, no open eaves and a kitchen outside the main houses. Some factors presented in the conceptual model were not collected in our study and could not be assessed. Variable "survey" was excluded from the vector density analysis due to its high correlation with meteorological data, i.e. temperatures and rainfall. However, this variable was included in the prevalence model and represents the possible time related variables, influencing prevalence, not covered by the parameters included in the vector density and prevalence analyses.

Table 18: Dependant and predictor variables introduced in the CART analysis.

Dependant Variable	Predictor Variables
Anopheles density	Current monthly rainfall (mm) Lagged monthly rainfall: one month (mm) Lagged monthly rainfall: two months (mm) Lagged average monthly minimum T°: one month (°C) Lagged average monthly minimum T°: two months (°C) Lagged average monthly maximum T°: one month (°C) Lagged average monthly maximum T°: two months (°C) Distance to marsh (m) • ≤ 300 • 301-500 • 501-700 • 701-900 • 901-1100 • > 1100 Altitude houses (m) • ≤ 1450 • 1451-1500 • 1501-1550 • 1551-1600 • 1601-1650 • > 1650 Type of crop in the marsh • Two crops/ year: rice and vegetable • Rice field • Vegetable • Few crop Housing : Poor constructions to better (4 categories) Keep livestock in the houses (yes/no) Houses in sprayed areas (yes/no) Use of insecticide treated nets (yes/no)
Positive slide	Age (year) Sexe Survey *Anopheles* density (number/houses) Past treatment during the 3 previous months (yes/no) Proxy for infectious bite (*Anopheles* density x SR) Keep livestock in the houses (yes/no) Sleep under a net (yes/no) Type of houses: Poor constructions to better (4 categories)

According to their overall discriminatory power, monthly rainfalls in the current month and with one month time lag emerged as the two strongest predictors for *Anopheles* density, followed in decreasing order of importance by spraying, net use, monthly minimum temperature with one and two months time lag, distance to the marsh, altitude of houses, and two months lagged rainfall (Table 19). The resulting regression tree is presented in Figure 19.

Every important predictor divides a node in two sub-nodes, either with a higher or a lower *Anopheles* density. A one month lag minimum temperature with a cut off point of 14.05°C was the first and most important splitter, with temperature below 14.05°C being associated with the lowest *Anopheles* density (mean: 1.6/house). This sub-node was further splitted by the variable "distance to the marshes". The highest *Anopheles* density (2.4/house) was found in houses located within 500 metres from the marsh. Spraying was then the best discriminative variables for this node, *Anopheles* density being the highest (3.4/house) when houses were not in treated areas. In this group, current rainfall had an influence over the average *Anopheles* density. Monthly rainfalls higher than 96.2 mm was associated with lower *Anopheles* density (2.2/house) than precipitations under this threshold (6.8/house). Monthly rainfalls in the preceding month (power: 99.9), net used (power: 83.1) and a lag minimum temperature of two months (power: 73.0) did not appeared as main splitters in the final tree whereas they were identified as important risk factors for *Anopheles* density as shown by the ranking of their discriminatory power (Table 19). This happens because they enter the tree as important surrogates in many nodes but never as main splitters.

The overall most important predictor variables for malaria infection are presented in Table 20. *Anopheles* density was the first splitter in the classification tree (Figure 20) with higher prevalence (46.3%) in houses with more than one *Anopheles* compared to houses with fewer *Anopheles* (prevalence: 26.5%). Then in this last group, parasite prevalence was lower in surveys 5 to 11 (year 2004 to 2007) compared to surveys 1 to 4. For surveys 1 to 4, age was the best discriminating factor with a threshold at 38 years; for survey 5 to 11, malaria prevalence among people leaving in the poorest houses was higher (25.7%) than people leaving in the better constructed ones (16.8%).

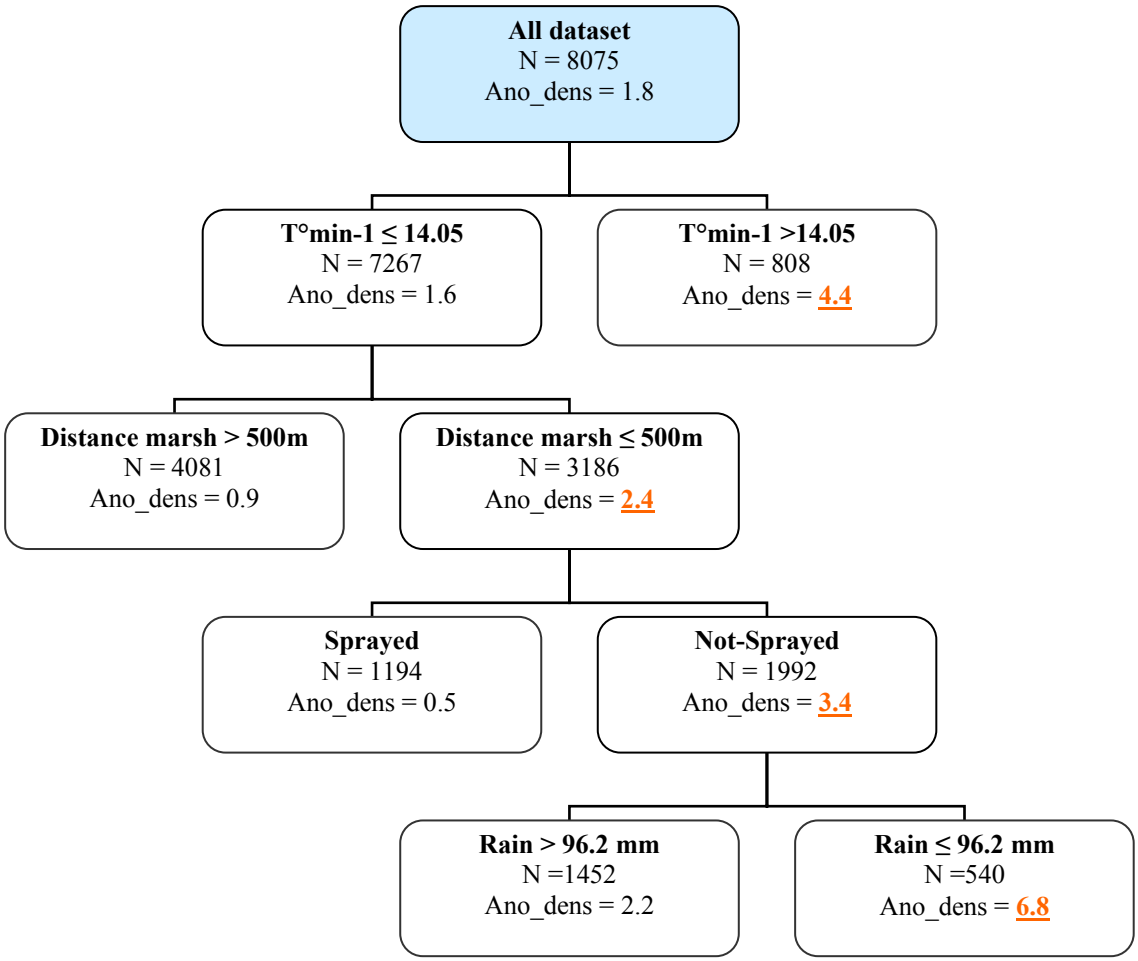


Figure 19: Regression trees representing the important risk factors for the *Anopheles* density per/house (Ano_dens).

The selected splitting variable is shown in each node in bold except for the first node

Table 19: Ranking of predictor variables for *Anopheles* density by their overall power as discriminant

Variables	Power
Rain	100
Rain - 1 month	99.9
Spraying	96.7
ITN use	83.1
T°min - 1 month	75.7
T°min - 2 months	73.0
Distance from the marsh	42.3
Altitude of the houses	31.9
Rain - 2 months	22.4
Type of crop	9.3
T°max - 1 month	5.7
T°max - 2 months	5.1
Housing	0.0
Livestock in houses	0.0

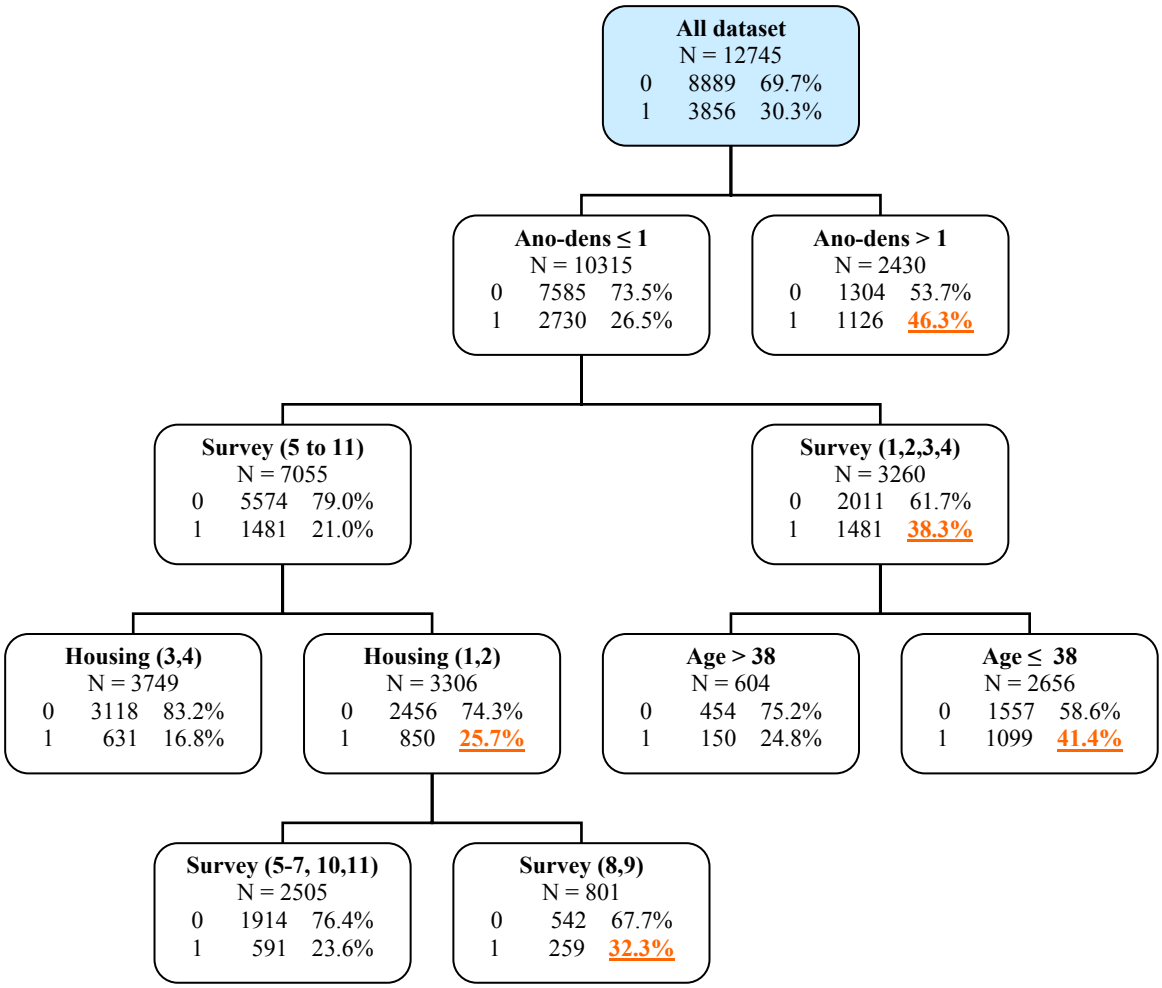


Figure 20: Classification trees representing the important risk factors for malaria prevalence. In each node 0 stands for negative slide and 1 for positive slide.

Table 20: Ranking of predictor variables for malaria prevalence by their overall power as discriminant.

Variables	Power
Survey	100
Anopheles density	91.0
Housing	23.4
Age	22.8
Treatment	5.8
Infectious bite	1.0
Livestock in houses	0.9
Sleep under a net	0.0
Sex	0.0

7.5. Discussion

The CART analysis is a new approach to assess the interplay of malaria risk factors and has been first employed by Thang et al [50]. Indeed, the CART method is a useful tool to deal with a large dataset and to explore the relationship and the relative importance between many variables as well as all their possible interactions. This is a major advantage over the classical multivariate analysis which cannot handle multiple interactions and do not rank the different explanatory variables by order of importance.

The factors influencing higher *Anopheles* density in the highlands of Burundi are in agreement with other studies, i.e. lower rainfalls [25,32,33], untreated houses [40] higher minimum temperatures [18,51], and breeding sites proximity [32,33]. In Karuzi, high rainfalls, ongoing or during the previous month, have a negative effect on vector densities. Minimum temperatures with 1 or 2 months time lag are good predictors of vector density, while housing conditions and livestock in houses have no predictive value. This last finding could be explained by the large predominance of the very anthropophilic vector *An. gambiae* s.s. (98.2% of the complex) [14] and could be different in presence of higher density of *An. arabiensis*. It is also obvious that vector control (ITN or IRS) reduces vector density in treated houses.

In this setting, *Anopheles* density (95% of the collected *Anopheles* mosquitoes are malaria vectors [14]) is one of the best predictor for malaria infection. This was also observed in other unstable transmission areas of Kenya and Madagascar highlands [5]. Indeed, in these countries the introduction of irrigated rice fields increased the number of breeding sites available and exposed the non-immune population to higher transmission [5]. Nevertheless, this is not always obvious, as sometimes very high vector densities may result in a very low vectorial capacity [52]. In our study, important variation in prevalence between surveys was observed and cannot be explained by any of the factors included in the analysis. Some variables such as improved health access, health status, and migration were not collected during the surveys. Differences between the first four surveys (year 2002 and 2003) and the later ones could be attributed to the normal decline of malaria prevalence after the malaria epidemic of 2001 [53] and/or to the introduction of the ACT and Rapid Diagnostic Test (RDT) in December 2003 and/or the subvention of the drugs by the MoH or any other changes that were not recorded. Mosquitoes and blood samples were collected at the same

time. However, when considering the time needed for the parasite to develop in the vector and the human host, postponed parasitological surveys by about one month could have improved the predicting power of *Anopheles* density on malaria prevalence. It is surprising that ITNs use was not associated with lower malaria prevalence while it affects the *Anopheles* density, a good predictor of malaria infection. This finding has been explained in a previous paper [14]. ITNs could not have any impact on malaria transmission because this had already been dramatically reduced by IRS.

This paper shows the importance of having an adequate malariometric database to better understand the malaria situation. For instance, it is commonly admitted that high rainfalls trigger malaria epidemic whereas in Burundi lower precipitations were associated with high *Anopheles* density, a powerful predictor of malaria infection. The long dry season preceding the epidemic of 2000 [53] may thus partially explain the outbreak. A more careful monitoring of the impact of rainfall and temperature variability on malaria should be further evaluated and a threshold risk set up in different areas. If this rainfall variability could be assessed a few weeks in advance, the follow up of the residual *Anopheles* densities when rainfall is low could be helpful in predicting or early detecting epidemics as presented by Lindblade et *al*. [54]. However, the practical use of these data for early warning and especially the initiation of expansive control measures based on it need to be assessed especially in the highlands [55].

In conclusion, it is essential to collect potential factors determining malaria in various settings, as differences exist between highlands. Furthermore, the CART method can refine initial conceptual model to each context by ranking the collected factors according their impact on malaria and consequently help to improve the monitoring of the most important one.

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Chapter 8. General discussion

Highland malaria & epidemics. Highland malaria is an increasing problem in Burundi and in several other countries. In the fifties, implementation of large scale indoor residual spraying has seen in Africa an important reduction of malaria in areas of unstable transmission particularly in the highlands [1-3]. However, after the "eradication period", vector control activities were stopped in most of the African countries and malaria has regained its past distribution. Since then, several deadly epidemics have been reported in the highlands [2,4-6]. In Burundi, the most important malaria outbreak ever recorded hit the central high plateaus in 2000 [7]. Despite the deterioration of the national malaria control programme other factors have been incriminated for this resurgence. The demographic pressure has increased from an average of 86 habitants/km² in 1950 to more than 300 habitants/km² in 2007 resulting in important environmental changes such as deforestation, irrigation, and swamp drainage for cultural activities creating favorable breeding sites for malaria vectors [3]. Furthermore, migration, collapsing health services, and increase of drug resistance have favored malaria in the unstable areas.

The management and prevention of these epidemics are key components of the Roll Back Malaria (RBM) initiative and different strategies are promoted [8]. Long-range forecasting (LRF), malaria early warning system (MEWS) and early detection systems (EDS) provide information with increasing accuracy at the level of a continent, a country, district and locality but with decreasing lead times from 10-14 months in LRF to 1-2 weeks for EDS [8]. Numerous models have been presented in the past years based on climate data and population susceptibility with varying reliability. Although they are more accurate in the desert fringes [9,10], they seem less reliable in the highlands where interaction between climate and malaria transmission is more complex [11,12]. Furthermore, many countries do not have the capacity to implement LRF and MEWS and the priority should be given to improve surveillance and case management to allow early detection and control of epidemics within 2 weeks of onset [13].

The 2000 epidemic in Burundi has highlighted the limits of the Ministry of Health and of the international NGO's to early detect and control epidemics. The low number and unprepared medical staff, a nutritional emergency at the same time, the absence of outbreak preparedness

and response plans were responsible for the delay to recognize and start the control measures in Karuzi (Chapter 3) and in other highland provinces [14]. Furthermore, effective case management could not be reached due to the high resistance level against Chloroquine and Sulphadoxine-Pyrimethamine [15]. The only remaining strategy was to implement vector control activities in order to stop the transmission. With this intervention we demonstrated that IRS and provision of ITNs were feasible in an open setting during complex emergencies, but failed to control the epidemic because it was initiated too late. Indeed the malaria transmission probably stopped before the epidemic had been declared. For the future, an early detection and control plan should be available. In every highland province of Burundi, two or three health centres with laboratory facilities could be selected as sentinel surveillance sites. District or provincial health authorities should be trained to analyze the data and to launch an alert when the epidemic threshold is reached [16]. Emergency stock of drugs, diagnostics and other materials must be available to respond quickly after the detection of the epidemic. Parts of these measures have been implemented in Burundi. National weekly collection of malaria cases have been set up in all health facilities (2001), a new protocol with the effective drugs Artesunate-Amodiaquine (ACT) was adopted in 2003, and a national strategy to prevent, to detect and to control epidemics was initiated in 2004 [17]. However major improvement is required as in practice, the processing of the weekly data is not rapid enough to allow effective detection and subsequent control.

Potential factors that could influence transmission have been studied to better understand the malaria epidemiology in the highlands of Burundi (Chapter 7). Lower rainfall (current and with one month lag), absence of vector control measures (spraying and nets), higher minimum temperature (with one and two months lag), and breeding sites close to houses were associated by order of importance to high *Anopheles* density. Then *Anopheles* density (> 1 Anopheline/house), poor housing conditions and age below 38 years were associated with high malaria prevalence. The unusual relation between low rainfall and *Anopheles* density may than partially explain the epidemic that occurred in 2000 which was preceeded by an exceptional long dry season. We observed that climate, as in other highlands, has an important impact on malaria. High precipitations have a direct impact on breeding sites availability and vectors density. In Ethiopia [18] and Uganda [4,19], they were associated with malaria epidemic whereas in Tanzania, as found in Burundi, fewer cases of malaria were observed than the previous years [20]. To explain this last finding Paajimans and al. [21] observed that rain showers can flush away and kill the mosquito larvae and consequently

affect malaria transmission. Furthemore, rise in temperature has increased malaria distribution in several regions [22-25]. In Burundi highlands rainfall and temperature variability seems to have an important impact on vectors. A more careful monitoring of the impact of these meteorological indicators on malaria should be further evaluated and a threshold risk set up in different areas. If this rainfall variability could be assessed a few weeks in advance they could give enough lead time to implement emergency control measures. However, the practical use of these data for early warning and especially the initiation of expansive control measures based on it need to be further evaluated in the highlands [11].

The impact of non-climate related risk factors found in Burundi was also observed in other highland settings. In Kenya, *Anopheles* density was negatively correlated with the increase distance between breeding sites and houses [26,27]. Vector control measures, such as Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs), had a direct impact on the transmission in reducing *Anopheles* density and sporozoite rates [28-30]. The socio-economic status has an impact on the type of housing and higher number of mosquitoes was found in poorly constructed houses than better ones in the Ethiopian highlands [31]. However, other possible indicators (health access, health status, migration, vectors density during the preceding month) that could possibly influence malaria have not been assessed in our study and could explain the variability in prevalence between surveys. A good understanding of factors influencing malaria in different highland countries is necessary to implement more rational control strategies focusing on the most important ones.

Increase endemicity in the highlands. The maximal altitude of malaria has been reported between 1800 and 2000 meters [32] but some rare epidemics were observed at higher altitudes [18,33]. In epidemic prone areas, all the age groups are normally at risk and the transmission is restricted to a short period of the year. In this context MEWS has been favored and regular vector control programmes are seen as a waste of resources by some authors [11]. However an increase in malaria endemicity has been observed in Burundi and previous non-endemic or epidemic prone areas have become meso-endemic. In Karuzi, the high prevalence in 2 to 9 year old children (32.1% to 53.4%) and the high proportion of asymptomatic carriers show a shift in endemicity, with a more stable transmission (chapter 5). This has also been observed in neighboring highland countries [32,34]. Following this change, vector control should become part of the prevention measures to reduce malaria transmission also in the highlands. However the spatial and temporal distribution of malaria transmission should be assessed to

better target the control measures [35]. In several highlands, the clustering of mosquitoes around their breeding sites is higher than in lowland areas due to the low temperatures and the steep valley slopes [36-38]. In Burundi, 90% of the *Anopheles* population was found within 700 meters from the valley where are confined the most important breeding sites, and people here were also three times more at risk to contract malaria than habitants living in the higher part of the hill (Chapter 4 & 5). In addition, the transmission period in the highlands remains seasonal and occurs during periods more favorable for *Anopheles* and parasite development, usually during the hottest months and at the end of the rainy seasons.

IRS & ITNs. Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) are two vector control methods widely used with high efficacy against the *Anopheles*, malaria transmission, and malaria morbidity and mortality especially in the lower transmission areas [30,39,40]. ITN appears to be an excellent tool to control malaria in Africa, and 45 African countries have now included ITNs in their national malaria control strategy [41]. However evaluation of the ITN coverage is difficult and by 2005, the only countries that met the Abuja target, of at least 60% of the children aged<5 years sleeping under a treated net, was Eritrea [42]. One of the main constraints to reach the target is the regular re-treatment of the nets, which is usually low. The recently developed long lasting insecticidal nets (LNs) that are wash resistant and last the useful life of the net, is a major advance for the scaling up of nets. In Burundi, the systematic free provision of ITNs (80% being LNs) to pregnant women and children aged<5 years started only in 2005 via antenatal care clinics and expanded immunization programme. In one year, the coverage has been increasing from less than 6% to more than 25% for pregnant women in provinces supported by NGO's or UNICEF. Coverage implies the effective use of an ITN and not only the availability in the household. Although these data are rather far from the Abuja target, the new Global Fund round will probably boost ITNs implementation. Coverage in other provinces remains unknown. During the next round of the Global Fund, emphasis and a higher budget should be put on sensibilisation and better monitoring of the ITN distribution via the health system. However, nowadays coverage of at least 35% of the population and not only the most vulnerable groups is recommended to achieve an equitable community wide benefit [43].

The world eradication programme launched in the fifties has clearly demonstrated that, long-term use of IRS could markedly reduce the malaria burden, with an even greater impact in areas of unstable transmission. However, today only half of the African countries include IRS

in their national malaria control strategy [41]. Costs and environmental concerns are the main reasons why IRS is not used to its full potential. Many countries are reluctant to use DDT the cheapest insecticide available on the market due to fears of its effect on human health and on environment. However, the Stockholm Convention on Persistent Organic Pollutants of 2001 (http://www.pops.int/documents/convtext/convtext_en.pdf) approved the use of DDT for vector control but not for agriculture. Furthemore, WHO has recently re-endorsed the use of DDT to scale up IRS [44] reviving the intense debates between scientists sustaining or against the ban of DDT [45-51]. However the use of this chemical should be carefully considered in a case by case basis taking into account the pro and cons [52]. DDT is still the cheapest insecticide available on the market although the difference with other insecticides appears to be declining [53]. DDT has also the longest efficacy 6 to 12 months compared to the other class of insecticide reducing the number of application on houses. The acute health toxicity is minor. However its long persistence and the accumulation in body fat raises some concern about possible human health impact, despite the fact that studies show conflicting conclusions. On the other hand, different constraints restrict the use of DDT. First, the reduce usage of DDT in public health began after the emergence of DDT resistant mosquitoes and evaluation should be done before the implementation of this insecticide for IRS. Secondly, the illicit diversion of DDT from disease prevention to agricultural activities is a major concern. Finally, the patent of DDT has expired, explaining the lower cost but also raising concern about the quality of DDT available on the market [54]. In conclusion, DDT re-use have been promoted due to its long term efficacy and price, however the relative cost efficacy of the available insecticides (pyrethroids, carbamate, organophophate and DDT) will depend on geographical location, ease of access and quality, the habit of the vectors in each areas, the degree of vector resistance to various insecticides, and finally the customs and compliance of the population. Furthermore, in order to reduce the cost, use of IRS should be more rationale than it has been in the past.

In Burundi, during four years an intervention combining IRS and LNs has targeted the people living within 700 meters form the valley bottom and this before the main transmission period. The idea was also to create with the sprayed houses a shield allowing the population living higher in the corresponding non-treated hills to be protected. This spatial targeted intervention drastically reduced the vector populations by 82% (95% CI: 69-90) in the treated valleys. Similarly, transmission decreased by 90% (95% CI: 63%-97%, p = 0.001) (Chapter 4). Children from 1 to 9 years old in the treated valleys had a lower risk of malaria infection (Odd

Ratio: 0.55), high parasite density (OR: 0.48) and clinical malaria (OR: 0.57). Impact on malaria prevalence was even higher in infants with a reduction of malaria prevalence reaching 86% (OR: 0.14) (Chapter 5). The spraying in the valleys did not protect people living higher in the hills. However, only 10% of the transmission occurred at the top of the hills. The combination of both LNs and IRS has further reduced the *Anopheles* density, however, no additional impact on malaria transmission and prevalence was observed, the number of infectious *Anopheles* being already reduced to zero by the IRS. WHO has advocated the use of both methods. This will be probably useful only when the coverage of one or the other measure is not optimal as observed in Equatorial Guinea [55]. In conclusion, the targeted IRS was very effective in preventing malaria and epidemics have not been detected during the project whereas some alert have been triggered in neighboring highland provinces. Such strategy could be implemented with success in other highland countries with similar environment and *Anopheles* behavior.

IRS and ITN are almost equal in efficacy [41]. But, some differences in effectiveness and cost-effectiveness have been observed according the local conditions [56,57]. The choice of one or the other methods or even the combination will depend on feasibility and existing infrastructures [58]. IRS rapidly reduces transmission and is particularly suitable in unstable and epidemic areas or during emergencies. It necessitates highly professional vector control services, a good planning and timing of the activities, and a strict management and logistics support [59,60] but the involvement of the community is low. When population is exposed to more stable malaria, ITNs especially if they are long lasting, are less demanding than IRS, however to be fully effective important involvement of the community is required to induce behavioral changes. The IRS was the best option in Burundi during this period of instability and of extreme poverty. Indeed, more than 90% of the targeted population could be covered each year with IRS. Whereas the coverage with LNs was also high after the distribution (78%), it decreased quickly to reach 31.2% after four years. In these poor housing conditions, the life span of the net was drastically reduced and a part of them were stolen. The national malaria control programme has now the required material and trained staff to implement this logistical complex IRS measure. It is important to keep this capacity and IRS could be applied in the epidemic areas when increase of malaria is observed or when rapid and high coverage is needed. However, in order to target the individuals most at risk such as pregnant women and children under 5, LNs are easier to implement. More recently, it has been shown that moderate coverage of 35% of the entire population will give more protection to vulnerable

groups than high coverage (<80%) restricted to children and pregnant women [43]. Therefore, with more substantial funding, all the population living in the high-risk areas of the highlands should also be covered reducing malaria transmission and subsequently preventing potential malaria epidemics.

Insecticide Resistance. As we mentioned ITN and IRS are the most important components to roll back malaria. However, these methods, especially ITNs, rely on the use of pyrethroid insecticides and emergence of pyrethroid resistance in vector populations is a major concern for the sustainability of malaria prevention in Africa. Resistance to pyrethroids in African vectors has become widespread in the African continent [61-66]. Metabolic based mechanisms and a mutation in the target site (knock down resistance: *kdr*) are responsible for pyrethroid resistance in *An. gambiae* s.l. [67,68].

In Karuzi, IRS with pyrethroids has probably led to selection for the *kdr* mutation (Chapter 6). *Kdr* was present before intervention in 1% of the *An. gambiae* s.l. and increased to 83% after six years. However high occurrence of *kdr* mutations was also observed in the neighbouring province far away from the intervention areas which suggest that other use of pyrethroids may have play a role in this fast selection. The *kdr* mutation is not always a good indicator of the resistance status of a mosquito and the possible reduction in efficacy of the vector control methods. In Burundi, pyrethroid resistance was only confirmed once *kdr* allele carriers reached 90%. Furthermore mechanisms, other than *kdr* could be held responsible.

The current recommendation for the impregnation of nets is the use of pyrethroids, leaving intervention vulnerable to failure if effective resistance is selected in the mosquitoes. While the choice of insecticide is wider for IRS with four classes registered (pyrethroids, organochlorides, carbamates, and organophosphates), resistance to each of these classes has already been observed in some areas [69,70]. There is an urgent need to identify new insecticides for both house spraying and treating nets. An encouraging initiative is the implementation of the Innovative Vector Control Consortium set up to develop new insecticides and which has received in 2005, 50 million US$ by the Bill and Melinda Gates foundation. However, it is likely to be at least a decade before this initiative manufacture any new alternatives. While waiting for these new products, insecticide resistance management should be implemented to keep the efficacy of the tools already available as long as possible. Rotation, mixtures or mosaic of different classes of insecticides with different modes of action

need to be further evaluated. The use of unrelated insecticides could be applied using different measures (e.g.: combination of ITNs and IRS) or within the same methods. Combining different classes of insecticides on the same net has already been tested with success [71]. The main constraint was the impregnations that need to be done by trained people and the production of long lasting nets should overcome this problem. Before implementation of any insecticide-based control activities, level of resistance in the local malaria vectors should be assessed. This will provide baseline information that will help to choose the best class of insecticide. Furthemore, WHO insecticide susceptibility bioassays should be done annually, in different areas, in order to monitor resistances in the country.

Case management & Intermittent Preventive Treatment. Access to affordable and effective antimalarial drugs is essential for an effective malaria-control programme. The Roll Back Malaria prone an effective treatment within 24 hours of the onset of symptoms to reduced the progression to severe disease, preventing high fatality rates. Drug resistance is incriminated as one of the causes of the high increase of malaria cases in numerous countries. However, change in drugs is a long winded process. In Burundi the first report of CQ resistance was in 1982 [72], more recently in vivo tests have been done in 2000 and also showed resistance to SP [15]. It is only in December 2003 that a combination of Artesunate and Amodiaquine (AS + AQ) was adopted by MoH for uncomplicated malaria cases in all the health facilities. Consultation, confirmed diagnosis by rapid tests or blood smears and full treatment are normally available at highly subsidized prices (free for children below 5 years old and pregnant women and 0.2 US$ for the rest of the population). A community based study conducted nine months after the introduction of AS and AQ in one of the Burundi province has shown that among children with probable malaria, only 60% sought formal care and the overall treatment coverage was low (14.1% treated with AS+AQ and 5% with quinine) [73]. However, no further studies have been carried out in other provinces to confirm this result and assess the availability and proper use of ACTs. Financial barriers to health care access (treatment given at a price ten times higher than normal price), inappropriate diagnosis and prescriptions could contribute to this low coverage. According to the Roll Back Malaria strategic plan, 80% of the people suffering of malaria should seek efficient treatment within 24 hours of the onset of symptoms by 2010.

Prescribing IPT to pregnant women has not been implemented in Burundi due to the high resistance against SP found in 2000 (between 10.9% to 52.8% according the MoH [15] and

66% reported in Karuzi by MSF) and WHO does not recommend its implementation if failure rate is over 50% [74]. However SP has not been used since 2003 and resistance against this drugs could have dropped. Burundi should assess the effects of IPTs with SP on pregnant women before deciding to include it in their national malaria control policy.

Control vs elimination. Recently there has been an expansion of ITNs and IRS coverage and an improved implementation of ACTs in African countries. Reduction of malaria mortality and morbidity is now becoming a reality in some of these areas [75-77]. These measures, coupled with increase coverage of IPT in pregnant women or introduction of IPTs for infants [78,79], could help to reach the target of RBM by 2010 (i.e: 80% of people at risk are protected, 80% of malaria patients are treated with effective drugs to ensure a 50% reduction in the burden of malaria compared to 2000 and 75% by 2015 [80]). However, substantial funding would be necessary to reach this objective. The cost for scaling up malaria control in Africa, between 2006 and 2015, is estimated around 17.4 billion US$ in the most optimistic scenario to more than 21.6 billion US$ for the worst [81]. Burundi has received by the global fund 17.8 million US$ during the past four years and will received an additional 33.7 millions for the next six years. This funding will help improve malaria control but additional funding will be necessary to reach the estimated 18.4 million US$ per year needed in Burundi to fulfill the RBM target [81].

Whereas the targets to control malaria are far from being reached in most of the African countries, malaria eradication is back on the agenda after the declaration of the Gate foundation in October 2007. In the past, attempts to eliminate malaria in hyper to holoendemic transmission areas has failed [77,82-84]. In contrast, successful elimination initiatives have been observed in less endemic areas in the highlands of Madagascar [85] and most of the South African territory [77]. However, without continuous control measures, some of these areas have experienced malaria resurgence. To achieve malaria elimination in areas of hypo to mesoendemic transmission, import of new malaria cases in these areas from their contiguous hyper and holoendemic regions should be prevented [86]. However, despite important success in some countries complete malaria elimination in Africa will not be possible with current knowledge, systems and tools available today. Sustained political commitments and financial resources, robust strategies, effective protective vaccines, new drugs and insecticides associated with current existing control measures, would be necessary to eliminate malaria in endemic areas. Furthermore, a successful malaria elimination

programme will require important investment to develop strong health care systems to be able to deliver antimalarial drugs or other interventions to those at risk in countries that often lack the most basic services.

Monitoring & evaluation. Monitoring and evaluation are essential components of the national malaria control programmes [87]. It ensures that activities are being well implemented and allows decision-makers to know all problems and constraints, which may prevent certain objectives from being reached. It also provides information that would help to re-orientate their planning. According the global malaria programme, monitoring is a continuous and regular process. It allows recording of the progress made by national health and malaria programmes. It should measure, through routine surveillance, household and community surveys, and health facility surveys, five global indicators; malaria death rate and malaria cases in high risk groups (children under 5 and other target group), availability and use of treated net, proportion of treatment given within the 24 hours, and availability of drugs in health facilities [41,87] and when implemented, IRS coverage.

Burundi has highly efficient and subsidized drugs, 80% of the population live within five kilometres from a health center. LNs are distributed to pregnant women and children under five through antenatal care and vaccination programmes. IRS is implemented punctually in epidemic regions. However, the coverage of ITNs is only known in few provinces and should be assessed in each province. The availability and proper use of ACTs should be also monitored regularly. Without an efficient health system and a well-developed health management information system, the systematic measurement of interventions coverage and impact are lacking. Investment in strengthening health systems, increasing the number of trained staff, improvement of the existing health information systems, and development of evaluation surveys and tools would help to collect more accurate information. Without them Burundi will never be able to evaluate its malaria control strategy and determine how far they are from their objectives.

In conclusion, major efforts have been done by the MoH to improve the control of malaria in Burundi during the past years. The targeted vector control programme in Karuzi has been successful in reducing malaria in the high-risk areas and could be extended within the countries and in other countries with similar topography, environment and vectors behavior. It has allowed improving knowledge about the epidemiology of malaria in the highlands and

strengthening the capacities of the health authorities to implement and follow complex intervention measures and evaluation surveys. Despite a reduction of malaria since the 2000 epidemics, control of malaria to reach the RBM target by 2010 will require a general improvement in the health and surveillance systems. With sustained efforts in the interventions already started, such as the scaling up of IRS and/or LNs in the malaria high risk areas and the free access for children to health facilities, with the implementation of IPT's for pregnant women, and a general increase in the living condition of the population, malaria could be effectively controlled in Burundi especially in the highlands.

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Annexes

Am. J. Trop. Med. Hyg., 79(1), 2008, pp. 1–2

Editorial

Malaria Control in the Highlands of Burundi: An Important Success Story

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Throughout Africa, successful high-impact malaria control programs are being implemented that emphasize sound vector control as an essential component of integrated national malaria control programs. Most programs are using insecticide-treated bednets (ITNs) and/or indoor residual spraying with insecticides (IRS). There are important considerations regarding which technique or combinations of the two work best for different epidemiologic situations and whether additional vector control measures are necessary to achieve significant reductions in malaria morbidity and mortality.

In this issue, Protopopoff and others present their paper on "Spatial targeted vector control is able to reduce malaria prevalence in the highlands of Burundi." This is a landmark paper because it describes how fundamental information on key differences in patterns of transmission within the highland environment in Karuzi Province should be used to plan and guide appropriate targeted interventions. Given that > 90% of malaria transmission occurs in the low-lying valleys, the 4-year study targeted all control vector control measures to these areas, covering ~90% of the houses with an annual round of IRS and one round of bednet distribution. Significantly, after the targeted intervention was compared with control valleys, this study showed a marked reduction in three important malaria indicators: malaria prevalence, high parasite density, and clinical malaria. A companion paper by Protopopoff and others,[2] published in the *Malaria Journal*, describes the impact of the intervention on reductions in vector populations and malaria transmission. *Anopheles* vector densities were reduced by 82% and transmission intensity was reduced by 90% in the intervention areas.

Together, these two integrated studies[1,2] show the potential value of targeted vector control for addressing the problem of malaria in highland areas of Africa. A strong case is made that epidemics can be managed and even prevented if continuous vector control interventions, in combination with effective case management, are applied to areas within the highland environment where transmission is most intense and focally distributed. Interestingly, the studies showed that the IRS component of the intervention had the most dramatic impacts on vector densities, malaria transmission intensity, and corresponding epidemiologic indicators of malaria infection and disease. Although the bednets served to reduce vector densities, there was no additional reduction in transmission intensities. Similar findings have been reported in Eritrea.[3] The absence of additional impact of nets over IRS is probably because of the high coverage achieved with IRS. The combination of both methods should not be excluded when full coverage with IRS is not optimal or difficult to sustain over time. Given relationships between intensities of transmission and malaria prevalence in Africa,[4] at least for the findings in Karuzi Province, Burundi, it seems that the strategy of using IRS as the predominant method for vector control served to reduce transmission intensity to levels less than one infective bite per person per year for most of the populations covered by the intervention. Otherwise, the marked reductions in malaria prevalence would not have been seen.

The scientific findings for the highland malaria control interventions in Burundi are yet another good example of malaria control success stories. Barat[5] provides an account of four other recent and highly notable malaria success stories, in Eritrea, Brazil, India, and Viet Nam. According to Barat,[5] successful malaria control programs share the following characteristics: "conducive country conditions, a targeted technical approach using a package of effective tools, data-driven decision-making, active leadership of the government, involvement of communities, decentralized implementation and control of finances, skilled technical and managerial capacity at the national and sub-national levels, hands-on technical and programmatic support from partner agencies, and sufficient and flexible financing." In the long road ahead for effective malaria control, it is vitally important that national malaria control programs make their findings known through peer-reviewed publications. Success stories at the national levels, especially dramatic findings from environmentally specific results such as the highland areas in the reports by Protopopoff and others,[1,2] make a valuable contribution to the global fight against malaria. In perspective, malariologists should not have to keep referring to the Garki Project[6] as the hallmark of malaria control. There are plenty of recent successful national malaria control program accomplishments that need to be documented and publicized so that we can all learn what works and what does not under the diverse environments in which malaria still thrives.

Are malaria vector control approaches involving IRS and ITNs sufficient? Even after the intervention in the study by Protopopoff and others,[1] malaria prevalence was still > 30% in the intervention areas. By all public health standards, this high prevalence is still unacceptable. If IRS and ITNs cannot do a better job of reducing transmission, this begs the question of what additional vector control measures need to be considered? There is a very strong case for implementing larval control through environmental management and the use of bio-insecticides to treat those larval habitats that cannot be eliminated. However, there are too few demonstrations that the additional measures, beyond IRS and ITNs, to target immature stages of anopheline mosquitoes have a substantial impact on malaria transmission and resulting morbidity and mortality. This is one reason why integrated vector management strategies (IVMs) have not been fully embraced and implemented in public health–driven malaria control programs. Clearly, more comprehensive IVM strategies and tools are going to be required as successful malaria control programs in Africa and elsewhere transition their program objectives to strategies for country-wide malaria elimination.

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