# STUDY OF MALARIA VECTORS BEHAVIOUR IN THE SOMALI CONTEXT

10 October 2014

by:
Dr. Mawlouth Diallo
WHO consultant

Co-Investigators:

1. Dr. Jamal Amran, Medical Officer_WHO/Somalia
2. Mr. Said Dahir Ali Ent_FP Somaliland & Somalia
3. Mr. Abdulhafid Hussen Yassin En_FP, Puntland
4. Dr. Jeylani Busuri Mio En_FP, South Central Zone

# STUDY OF MALARIA VECTORS BEHAVIOUR IN THE SOMALI CONTEXT

Design, Layout and Printing: UNON Publishing Services Section – ISO 14001:2004-certified

# Table of Contents

# ACKNOWLEDGEMENT

We gratefully acknowledge the following persons and institutions for their co-operations and assistance.

- The authorities of the Ministry of Health of Somaliland, Puntland and South Central Zone, especially Dr. Abdi Abdilahi Ali NMCP Director in Somaliland, Dr. Abdulkarim Hussen Hassan, NMCP Director in Puntland and Dr.Abdulqani Sh. Omar NMCP Director of the South Central Zone
- Dr. Jamal Amran, Medical Officer, WHO/Somalia, who initiated the study for his leadership.
- WHO members of staff in Hargesia Office especially Fahmi Essa Yusuf, Data Manager and Dr. Abdkarim Mussa, at the Malaria Control & Elimination WHO Hargeisa Office for their constant assistance and for facilitating the organisation of the study
- WHO_EMRO staff members in Nairobi, for their support and assistance.

We acknowledge the assistance of the District Health Officer and Heath community members as well as the populations in all localities visited for their friendly disposition and support to the team.

# 1.0 INTRODUCTION

Malaria is a major public health problem in Africa. Despite more than a century of study, malaria continues to be a major public health concern throughout the world and especially in Africa where 90% of the global cases are recorded. The situation is even worsening with the spread of drug resistant parasites strains, increase of insecticide resistance in vector populations and deleterious economic status of exposed populations. The World Health Organization (WHO) estimates that there are about 300–500 million clinical malaria cases annually and that 1–1.5 million people die of malaria each year. *Anopheles arabiensis* and *Anopheles gambiae* are the principal vectors of malaria in sub-Saharan Africa, but in some areas *An. arabiensis* is better adapted to dry environments than *An. gambiae* (Lindsay et al. 1998). Knowledge of host-feeding pattern and resting behaviour of mosquito vectors are important for understanding the host- vector relationship and dynamic of disease transmission and for development of control strategies. For *A. gambiae*, it has been observed that its vector density and malaria transmission intensity display similar patterns in relation to environmental conditions such as rainfall and spatial and seasonal heterogeneity among shelters. Five species of plasmodium causes human malaria. Among these, *P. falciparum* is responsible for most of the mortality *P. Vivax* causes considerable morbidity and *P. malariae, P. knowlesi* and *P. ovale*, are less prevalent around the world. (Aslan *et al*.)

# 2.0 BACKGROUND

## 2.1 TOPOGRAPHY AND CLIMATE

Somalia is the eastern most country in Africa and its terrain consists mainly of plateaus and plains with a few highland areas in the northern margins [Hadden 2007]. In the far north are the east-west ranges of the Karkaar Mountains rising as high as 2440 m (Figure 1.1A). On the eastern and northern sides is the longest coastline in Africa. On the west is the vast Somali plateau consisting of series of tablelands. In the East and the South, the plateau ends in arid steppes, from under 200 meters at the Indian Ocean. The plains drain into the Juba River in the south and Shabelle River in the centre, which disappears into a swamp before reaching the coast (Figure 1).

Most of Somalia receives less than 500 mm of rain annually, and a large area encompassing the northeast and much of northern Somalia receives as little as 50 to 150 mm (Figure 2). The southwest receives 330 to 500 mm.

During the 2013, except some pockets with depressed rainfall, field records indicated significant amount of rains in almost all regions of Somalia including those selected for this VBS study (Table 1; Source of data: SWALIM/FAO)

**Figure 1:** altitude map showing the three zones of Somalia

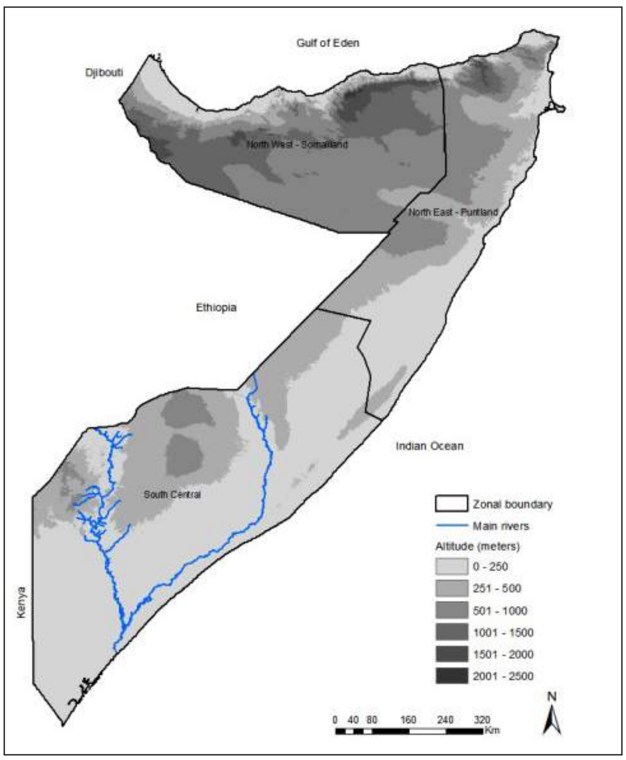



**Figure 2:** mean annual precipitation map of Somalia

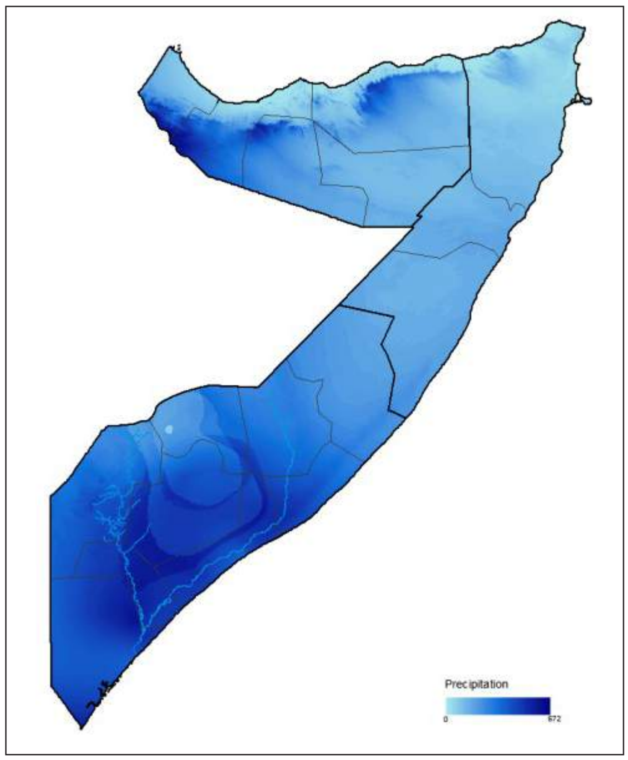


**Table 1: Monthly precipitation recorded in the different regions selected for the study in 2013**

| Location | Jan | Feb | March | Apr | may | jun | Jul | Aug | sep | Oct | Nov | Dec | Total | Rain days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gabilley | 0 | 0 | 55.5 | 10.5 | 103 | 27 | 81 | 84 | 41.5 | 16 | 43 | 0 | 461.5 | 41 |
| Berbera | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 195 | 25 | 223 | 6 |
| Galkayo | 0 | 32 | 55 | 25 | 0 | 0 | 0 | 0 | 0 | 91.5 | 50 | 0 | 253.5 | 16 |
| Burtinle | 0 | 0 | 20 | 7 | 45 | 0 | 0 | 0 | 23 | 19 | 29 | 0 | 143 | 14 |
| Bossasso | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 35 | 0 | 55 | 3 |
| Jowhar | 0 | 0 | 56 | 339 | 36 | 18 | 13 | 5 | 14 | 131 | 173 | 0 | 785 | 40 |
| Janale | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 118 | 0 | 111 | 253 | 8 |

Mean daily maximum temperatures throughout the country range from 30°C to 40°C, except at higher elevations and along the Indian Ocean coast. Mean daily minimum temperatures vary from 20°C to more than 30°C. Northern Somalia experiences the greatest temperature extremes, with readings ranging from below freezing in the highlands in December to more than 45°C in July in the coastal plain skirting the Gulf of Aden. The north's relative humidity ranges from about 40% in mid-afternoon to 85% at night, varying with the season. Temperatures in the south are less extreme, ranging from about 20°C to 40°C. The hottest months are February through April. The coastal zone's relative humidity usually remains about 70% even during the dry seasons. This diversity of climatic conditions from south to north has a marked effect on the distribution, abundance and infectivity of malaria by dominant vectors.

## 2.2 POLITICS AND GOVERNANCE

Between 1840 and 1886, the British East India Company signed a series of trade treaties with various Somali chiefs leading to the eventual establishment of the British Somaliland comprising present day Somaliland. Italy also marked out the Italian Somaliland in the South between 1897 and 1908 while Ethiopia claimed the Western Somaliland (Ogaden region) in 1897 [Fage & Olivier 1985; Hadden 2007; In the South, the Italian rule lasted until 1941, when this region became a British Protectorate during the Second World War until 1949, when the United Nations returned it to Italy as a trusteeship. Great Britain took a more hands-off approach to governance in Somaliland, leaving more responsibility in the hands of local leaders but also providing less by way of infrastructure while the Italians dedicated significant effort towards developing their colony. The British trusteeship over Somaliland ended in June 26 1960 when this region was declared independent. Shortly after, on July 1 1960, Somaliland and the South united to form the Republic of Somalia with Mogadishu as its Capital City

## 2.3 MALARIA EPIDEMIOLOGY IN SOMALIA

Published descriptions of malaria in Somalia began as early as the 1930s with reports on malaria in the Banadir region [Gelonesi 1931; 1932]. A malariometric survey undertaken between 1935 and 1936 suggests that *Plasmodium falciparum* prevalence of above 50% in several areas in southern Somalia [Cicchitto 1938]. In a report to the WHO in 1955 by a consultant entomologist, Dr Giglioli, several observations were made on the epidemiology of malaria in Somalia [WHO 1960].

- The arid northern and central parts of Somalia were considered to very low malaria risk.
- In these arid areas the likelihood of epidemics were associated with heavy rains while local endemic foci were likely to be around water collection points such as springs, wells and dams
- The coastal regions were considered malaria free.
- Endemic malaria was associated with the riverine regions in the south.
- Transmission was largely seasonal and therefore there were no areas of holo-endemic transmission.
- Little was known about the malaria epidemiology among nomadic pastoralists.

Epidemiological surveys undertaken mainly in the southern regions of Somalia by the National Malaria Service and the Italian Naval Medical Research Centre in the 1950s showed mean parasite prevalence of 2% to 10% in the upper, middle and lower Shabelle areas; 2% to 60% in the upper Juba region; and up to 20% in the middle and lower Juba [WHO 1960].

In 2008 an empirical map using just under 500 community parasite prevalence survey data from 2005 to 2007 was used to develop a malaria risk map at 5 x 5 km in Somalia using Bayesian geostatistical methods [Noor et al 2008]. This map revealed in the north of the country parasite prevalence was less than 2% with pockets of between 5% and 9%. In the South especially around the riverine areas, point estimates ranged from 0% to 52% with a median estimate of 5%. Similar patterns were observed in a revised map developed using about 1500 clusters from the period 2005 to 2009 with most parts of the country under prevalence of <5% [Snow et al 2009]. Entomological evidence shows that *Anopheles arabiensis* is the main and often, the only vector in the country [Maffi, 1958; 1960; Maffi & Colluzi, 1960; Mouchet *et al.*, 2004]. The presence of *An. merus* in Mogadishu has not been confirmed [Mouchet *et al.*, 2004]. *An. funestus* and *An. nili* have also been reported in the South [Maffi, 1958]. In the North East *An. pharoensis* and *An. d'thali* have been described [Choumara, 1961]. The two malaria seasons were identified as following the spring (May-August) and the autumn (December – January) rains.

A most recent study conducted by the US Armed Forces Pest Management Board in several districts belonging the three zones revealed the presence of 16 Anopheles species including the main malaria vectors in Africa *An. gambiae sensu lato*, *An. funestus*. According to this study, *Anopheles arabiensis* and *An. gambiae* are the principal vectors countrywide. *An. funestus* occurs in low altitude areas and *An. merus* in brackish coastal areas. Others Anophelines species include *An. azaniae, An. cinereus, An. coustani, An. daudi, An. demeilloni, An. dthali, An. macmahoni, An. paludis, An. pharoensis, An. pretoriensis, An. rhodesiensis, An. salbaii, An. sergentii*. The same study assumes that *An. arabiensis* breeds in ground pools, tanks, wells, water storage bags and other places. In the north it survives the dry season in covered water containers. *An. gambiae* requires cleaner, more extensive breeding areas, and hence occurs in rainy periods or wet areas and breeds in ground pools. This study assumes also an exophilic tendency of *An. arabiensis* while *An. gambiae* tend to stay indoor after biting. *An. funestus* is usually found along rivers and in permanent water.

The entire population of Somalia is at risk of malaria, with 54% at high risk. The intensity of malaria transmission varies in different parts of the country, ranging from unstable and epidemic- prone in the North East Zone (NEZ)/ Puntland and North West Zone (NWZ)/Somaliland, to moderate in Central Zone and moderate to high in the South Zone. Malaria remains the most common cause of illness and death in Somalia, particularly among pregnant women and children under the age of five. During 2012, malaria trend continued to decrease (9500 malaria cases in 2010 vs 8 8 49000 8 in 2006 reported in Somalia88), as a result of a coordinated effort of WHO, health authorities and partners against the disease. At the core of the intervention was the strengthening of the detection system using Rapid Diagnostic Test (RDT), followed by prompt and effective treatment with artemisinin-based combination therapies. Prevention activities were also scaled up to control malaria morbidity by distribution of lasting insecticides-treated bed net to protect pregnant women and children. These interventions were sustained by the introduction of vector control using Indoor Residual Spraying with insecticide (IRS).

However, this intervention faces up to many challenges that limit its success across the country (all 3 zones included). One of the first shortcomings of its implementation is related to the structure of the households in some communities mainly composed by nomadic populations. These communities represents more than 60% of the total population and are constantly moving over long distances looking for pastureland due to the erratic pattern of rainfall. Therefore they are living in temporary dwellings remove at each departure. The scarcity or absence of data concerning the vectors behaviour in the different biogeographic zones is also a major limitation.

# 3.0 PROBLEMATIC AND JUSTIFICATION OF THE PROJECT

Risk of exposure of human to infectious bites of vectors is not uniform at a bioclimatic level as well as in short distances at local level, and variations in the abundance, dispersal, biting and resting behaviour of mosquitoes occur in a given area, and over time. These variations impact on the level of malaria transmission but also on the current vector control strategies that have to come up against insecticide resistance that could be behavioural or physiological. Therefore the selection and application of vector control strategies of focused and timely interventions requires detailed information on the local entomological parameters. These parameters includes : i) the geographic distribution of the vectors, ii) the entomologic transmission indices mainly the entomological inoculation rate (EIR), an index that relates both the human-biting activity of the Anopheles vectors and the risk to humans of malaria infection, iii) temporal and spatial dynamic, iv) feeding pattern, biting and resting behaviour of the vectors that are of great practical importance in malaria transmission and control using insecticides (Sandoshom, 1980), v) vectors susceptibility to insecticides etc. *Anopheles arabiensis* and *Anopheles funestus* are involving in malaria transmission vectors in Somalia. These vectors can vary their exophlicity and endophlicity and can present a real problem in malaria control programme. However few data are available in Somalia concerning these malaria entomologic parameters essential to set up a good action plan for vector control to limit wastage of scarce resources on controlling species that do not transmit malaria parasites.

To fill this gap, a study has been conducted to investigate resting and biting behaviours of *An. gambiae* complex and *An. funestus* as well as other potential malaria vectors in several villages belonging to different biogeographic zones. It purposed to answer these following questions:, Where species of the *An. gambiae* complex and *An. funestus* as well as other potential malaria vectors are normally resting and biting. Are they endophilic or exophilic, endophagic or exophagic? If there any changes in resting behaviour of An. arabiensis and what is new?

The data that have been generated, were used to assess the need or not for alternative malaria vectors control strategies. Then the most effective control strategy will be recommended for the area to improve current prevention and control strategies.

# 4.0 OBJECTIVES OF THE STUDY

The aim of this study is to investigate the resting and biting behaviour of the species of the *An.* complex and *An. funestus,* other potential malaria vectors in 12 villages of Somalia and recommend appropriate vector control strategies.

The specific objectives were:

1. To Carry out an entomological survey in the selected localities to assess the malaria vector presence, distribution and density
2. To study the vector biology and potential role in malaria transmission

    a. To determine the resting behaviour (indoors/outdoors) of *An. arabiensis* & *An. funestus* in the 12 villages selected.
    b. To determine biting behaviour (outdoors/indoors) of the malaria vectors c. To determine human biting rate by indirect calculation

# 5.0 METHOD AND MATERIAL

## 5.1 STUDY AREA

The study area covered the three biogeographic zones of Somalia (SCZ, SL, PL). In each zone four villages have been selected based on: the presence and abundance of Anopheline mosquitoes, ecological similarities, houses type, livelihood activities, deficiency of spray operation, malarogenic potentials and vulnerable to influx of source of infection, the willingness of villagers to cooperate with the mosquito collection efforts and accessibility of the location.

The Figure 3 and table 2 below present the location of all villages selected in each district of the 3 zones.

**Figure 3: Map showing the geographic position of the village investigated**

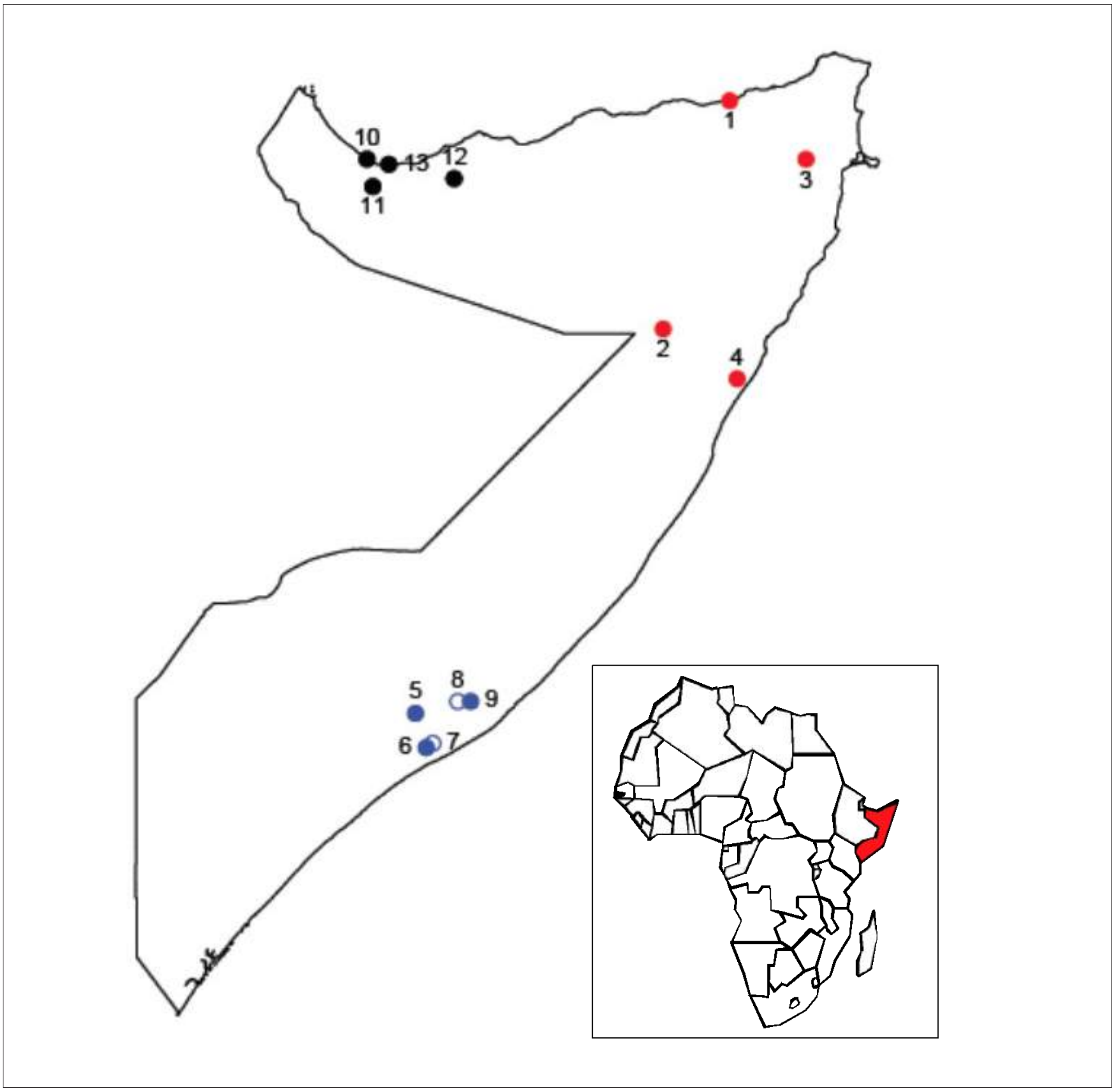


**Table 2: Research Areas with their respective villages and characteristic**

| Zone | Village | #HHs | Population | 2013 Rain (mm) | Intervention IRS - LLINs | Malaria prevalence |
|---|---|---|---|---|---|---|
| PuntLand | Biyokulule | | 14000 | 0 | IRS & LLINs | 0 |
| | Jariban | 5254 | 31525 | 0 | LLINs | 0 |
| | Iskushuban | 3500 | 21000 | 0 | LLINs | 0 |
| | Burtinle | 8491 | 50945 | 0 | LLINs | 0 |
| South Central Zone | Jambaluul | 2983 | 20881 | 41 | LLINS | |
| | Marerey | 2449 | 17143 | 41 | LLINS | |
| | Jowhar-Ely | 500 | 1200 | 301 | LLINs | |
| | Jowhar Moyki | 1000 | 7000 | 301 | LLINs | |
| | Wanla-Weyn | 2704 | 18928 | 0 | LLINs | |
| Somaliland | Agabar | 270 | 1755 | 0 | IRS | 0 |
| | Turka | 100 | 650 | 0 | LLINs | 0 |
| | Biyolay | 60 | 390 | 0 | LLINs | 0 |
| | Ali Hieyd | 150 | 975 | 0 | IRS | 0 |

## 5.2 STUDY DESIGN

Cross-sectional study has been conducted in the 12 villages (SL, PL&SCZ) selected according to the standards described above. In each village, houses were randomly selected and afterwards households for mosquito sampling.

## 5.3 SAMPLING METHODS

For collecting mosquitoes, CDC_Light traps (LT), window exit traps and indoor pyrethrum knockdown catches have been used to determine potential variation in the vector abundance, behaviour and vector status. A total of 42 houses have been investigated in each village for a total of 168 houses in each zone and each visit. To account for possible competition between sampling methods, within the same house, 3 groups of houses have been chosen in each village, and in each group, mosquitoes have been sampled during 4 nights by window exit trap and CDC Light trap and 3 days by pyrethrum spray catch. These 4 consecutive nights/days represented one replicate/round of collection. Two (2) round of sampling have been conducted in each zone and village from February 29th 2014 to March 31st 2014. In each house a form have been used for recording parameters like: date, village name, type of vector control strategy (LLINs/IRS/combined/none) implemented, house type (brick/mud), structure of the house, number of persons (children and adults) that slept inside the room of the house from where the collection was made, presence and type of animals.

- **Pyrethrum spray collections (PS**): The pyrethrum spray collection method has been used to sample indoor-resting mosquitoes to assess the endophilic density in selected bedrooms. This approach has been also used to indirectly estimate the Human Biting Rate (HBR). A total of 30 houses were randomly selected in each village for pyrethrum spray collection. Collection by PSC has been performed during 3 days between (6am-10am) and 10 houses were sprayed per day. In each house all bedrooms have been sprayed. Upon collection, Anopheline vectors as well as Culicines mosquitoes have been sorted, counted and morphologically identified following available keys. For the malaria vectors, blood-fed mosquitoes have been collected stored in tube containing a desiccant (silicagel) for subsequent determination of the host source. The origin of blood meals has been identified using an enzyme-linked immunosorbent assay (ELISA) using peroxidase- conjugated of antibodies (Beier et al. 1988). The antibodies have been selected based on the domestic animals prevalent in the study villages including human. The human biting rate (HBR) have been estimated using the following formula : HBR = ( nm / ns) x IA with nm representing the number of mosquitoes collected, ns the total number of sleepers and AI the Anthropophilic index.

- **CDC light trap collections;** CDC light traps have been used to estimate the vector biting rates (number of bites per person per night) indoor and outdoor, for routine sampling and to provide additional data, including abundance, species composition. A total of 10 CDC light traps per day was set up to sample mosquitoes in 5 houses from each village; Five (5) light traps have been set up inside the bedroom and 5 light traps outside. In each House selected, a light trap has been set up in a bedroom near the sleeper protected by an untreated bed net to collect the biting mosquitoes. A second light trap was set up outside the same house in the courtyard, near a sleeper protected by an untreated bed net. When the team member was not able to stay on site until 18:00, the household owner has be shown how to switch on at 18:00 (sunset) and switch off at 06:00 (sunrise) and to tie the string around the collecting container before switching off, to prevent mosquitoes from escaping. Mosquitoes have been collected the next morning by the team members.

Mosquitoes were collected using a suction tube, killed frozen or using chloroform (in case freezer is not available) and placed in vials or petri dishes containing damp filter paper, covered and sealed with white tape. The petri dishes and vials were labelled accordingly using a permanent marker and placed in an ice box for transportation to the field laboratory where the mosquito specimens were processed.

- **Window/Exit traps collection (WE)**: Window Exit traps have been used to collect exiting mosquitoes in houses in each of the entomological sentinel sites and to estimate the exophilic tendency of the vector. A total of 7 window exit traps have been used per day to sample 7 houses selected in each village during 4 days of collection. Each trap have been fitted on a window or opening in each bedroom selected from 18:00. Only bedroom containing sleepers the night of collection have been used. The exit traps were collected the next morning at 6:00 am. The mosquitoes from each trap were collected using suction tubes and killed frozen or using chloroform (in case freezer is not available). They are then placed in vials or petri dishes containing damp filter paper, covered and sealed with white tape. The petri dishes and vials were labelled accordingly using a permanent marker and placed in an icebox for transportation to the field laboratory where the mosquito specimens were processed. All mosquitoes were transferred to the field laboratory for processing. These houses can be sampled for spray catches the next day after collection of traps.

- **Complementary Laboratory processing**. A random sample of at least 30 females belonging to the *An. gambiae* complex and collected indoor and outdoor in each village will be tested for species identification by PCR.

## 5.4 ENTOMOLOGIC TEAMS

**Somaliland**

- Dr. Abdi Abdilahi Ali NMCP Director
- Said Dahir Ali Entomology Focal Point (Team leader)
- Mohamud Hassan Adam Assistant Entomologist
- Ismail Muse Ahmed assistant Entomologist
- Abdirahman Mohamed Abdi assistant Entomologist

**Puntland**

- Dr. Abdulkarim Hussen Hassan, NMCP Director
- Mr. Abdihafid Yassin Hussein, Entomology Focal Point , (Team leader)
- Mohamed Ali Mohamed Ento. Assistant
- Abdulrashid Mohamed Adam Ento. Assistant
- Mohamed Ahmed Ali Ento. Assistant

**South Central Zone**

- Dr. Abdulqani Sh. Omar NMCP Director
- Dr. Jeylani Bussuri Mio Entomology FP (Team leader)
- Hassan Mohamed Jimcaale Ento.Assistant
- Mohamed Deq Sh.Diini

- Yousuf Osman Ooyow
- Mohamed Omar Bakar
- A/lahi Mohamed Muumin
- Omar Macalin Nourani
- Hassan Abdiyow Sh.Ali

# 6.0 ETHICAL APPROVAL

The protocol of the study has been submitted and approved by to the MoH and elders of each zone. It has been further carefully explained (the means of project, why it is undertaken and what would be the findings etc.) to the legal representatives of each village and community as well as each household owner to request their oral consent.

# 7.0 EXPECTED USES & USERS OF RESEARCH RESULTS

Direct a beneficiary is NMCP, will apply the project results a modelling tools in their routine activities and for providing appropriate vector control. An indirect beneficiary is community of target study areas through perception of vector resting behaviour.

# 8.0 DATA COLLECTION AND ANALYSES

Data have been collected and recorded by standard form of WHO slightly modified and thereafter into Microsoft Excel data base. Mosquito haves been identified in each field site by the corresponding Entomology FP. Entomological data have been analyzed using R Gui software (v.3.0.2). Means were compared using Kruskall-Wallis or Wilcox tests have been used to test difference between means of different collection among, round, traps, and villages. Significant levels were measured at 95% confidence level with significant differences recorded at $p<0.05$.

# 9.0 RESULTS

## 9.1. VECTOR BEHAVIOUR IN PUNTLAND

A total of 6564 mosquitoes have been collected in the 4 villages investigated including 4723 females and 1841 males (32 *An. funestus*, 70 *An. gambiae* and 1739 Culicines males). The table 3 shows the mosquitoes females collected using CDC_LT, WE and Spray catches in the domestic environment. The Culicines represented 90.38% of the mosquitoes and the Anopheles 9.61%. *An. gambiae* and *An. funestus* were the unique malaria vector collected. They represented respectively 3.77% and 5.84% of the fauna and further 39.2% and 60.79% of the Anophelines populations collected.

**Table 3 : Mosquito species females collected using the different sampling methods from February 28th 2014– March 31st 2014, in Puntland**

| Villages | Traps | Location | *An. gambiae* | *An. funestus* | Anophelines | Culicines | Total |
|---|---|---|---|---|---|---|---|
| | CDC_LT | In | 0 | 4 | 4 | 71 | 75 |
| | | Out | 0 | 0 | 0 | 28 | 28 |
| **Biyokulule** | Spray | In | 0 | 10 | 10 | 243 | 253 |
| | WE | Out | 0 | 1 | 1 | 49 | 50 |
| | **Sub Total** | | **0** | **15** | **15** | **391** | **406** |
| | CDC_LT | In | 7 | 0 | 7 | 480 | 487 |
| | | Out | 0 | 0 | 0 | 219 | 219 |
| **Buurtinle** | Spray | In | 2 | 0 | 2 | 323 | 325 |
| | WE | Out | 0 | 0 | 0 | 101 | 101 |
| | **Sub Total** | | **9** | **0** | **9** | **1123** | **1132** |
| | CDC_LT | In | 5 | 134 | 139 | 342 | 481 |
| | | Out | 6 | 114 | 120 | 436 | 556 |
| **Iskushuban** | Spray | In | 8 | 11 | 19 | 238 | 257 |
| | WE | Out | 0 | 0 | 0 | 80 | 80 |
| | **Sub Total** | | **19** | **259** | **278** | **1096** | **1374** |
| | CDC_LT | In | 123 | 0 | 123 | 532 | 655 |
| | | Out | 18 | 1 | 19 | 782 | 801 |
| **Jariiban** | Spray | In | 8 | 0 | 8 | 231 | 239 |
| | WE | Out | 1 | 1 | 2 | 114 | 116 |
| | **Sub Total** | | **150** | **2** | **152** | **1659** | **1811** |
| | **Total**<br>**%** | | **178**<br>**3.77** | **276**<br>**5.84** | **454**<br>**9.61** | **4269**<br>**90.38** | **4723**<br>**100** |

CDC_LT : CDC light Trap ; WE : Window Exit

### *9.1.1 RESTING BEHAVIOUR OF MALARIA VECTORS IN PUNTLAND*

A total of 39 mosquitoes have been collected using indoor pyrethrum sprays catches (SP) in 300 bedrooms belonging to 240 houses during the 2 the rounds of collection. Globally the indoor resting density was low in all localities investigated for the two malaria vectors and ranged from 0 to 0.14 female per bedrooms (f/br) (Table 4). This density was estimated of 0.13 for Anophelines, 3.13 (N=939) for the Culicines. For Anopheles species the mean densities were 0.07 f/br for *An. funestus* and 0.06 f/br for *An. gambiae*. The resting density of *An. funestus* was significantly higher than *An. gambiae* in Biokulule (p-value = 0.006783) while *An. gambiae* was more active indoor in Jariiban (p-value = 0.003621). For other locations the densities of both species were comparable.

This indoor resting density was very erratic and varied according to the round of collection and to the localities. The higher density has been recorded for *An. funestus* in Iskushuban (0.14 f/br) and for *An. gambiae* in Iskushuban and Jeriiban (0.13 f/br).

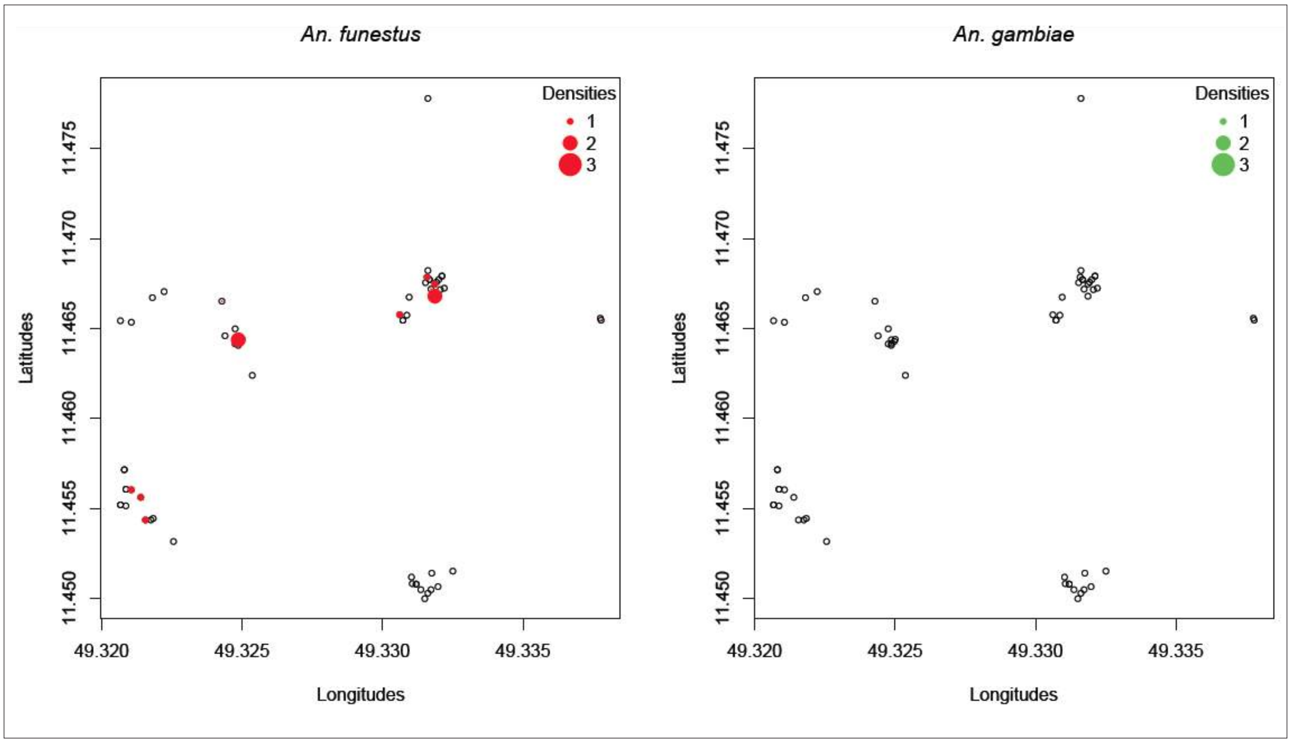
**Table 4 : Indoor resting densities for *An. gambiae* and *An. funestus* in different villages of Puntland (February 28th 2014– March 31st 2014)**

| | *An. gambiae* | | | *An. funestus* | | |
|---|---|---|---|---|---|---|
| **Villages** | **Number of specimens** | **Number of House** | **Densities (f/br)** | **Number of specimens** | **Number of House** | **Densities (f/br)** |
| Biyokulule | 0 | 79 | 0 | 10 | 79 | 0.13 |
| Iskushuban | 8 | 80 | 0.1 | 11 | 80 | 0.14 |
| Buurtinle | 2 | 71 | 0.03 | 0 | 71 | 0 |
| Jariiban | 8 | 70 | 0.11 | 0 | 70 | 0 |
| **Total** | **18** | **300** | **0.06** | **21** | **300** | **0.07** |

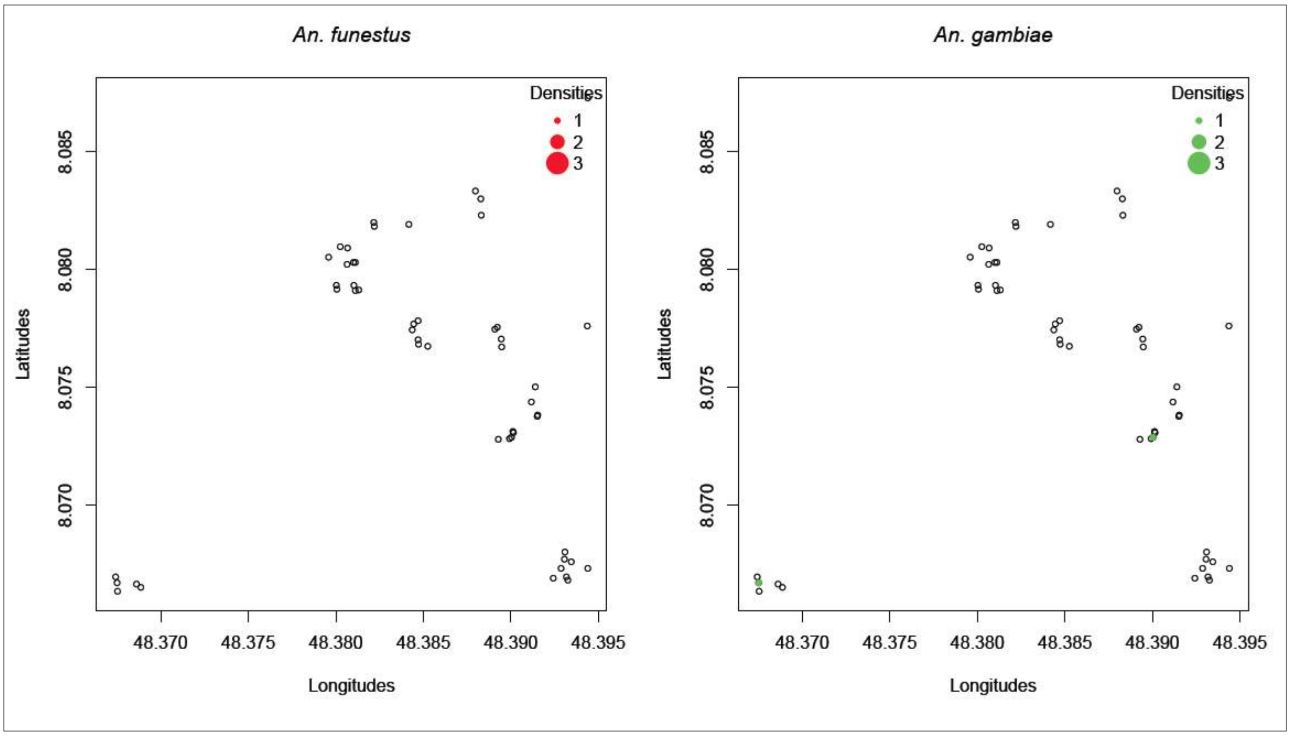
f/br : females per bedroom

The Map 1 show the geographic distribution of houses investigated in each village and those found associated with the malaria vector.

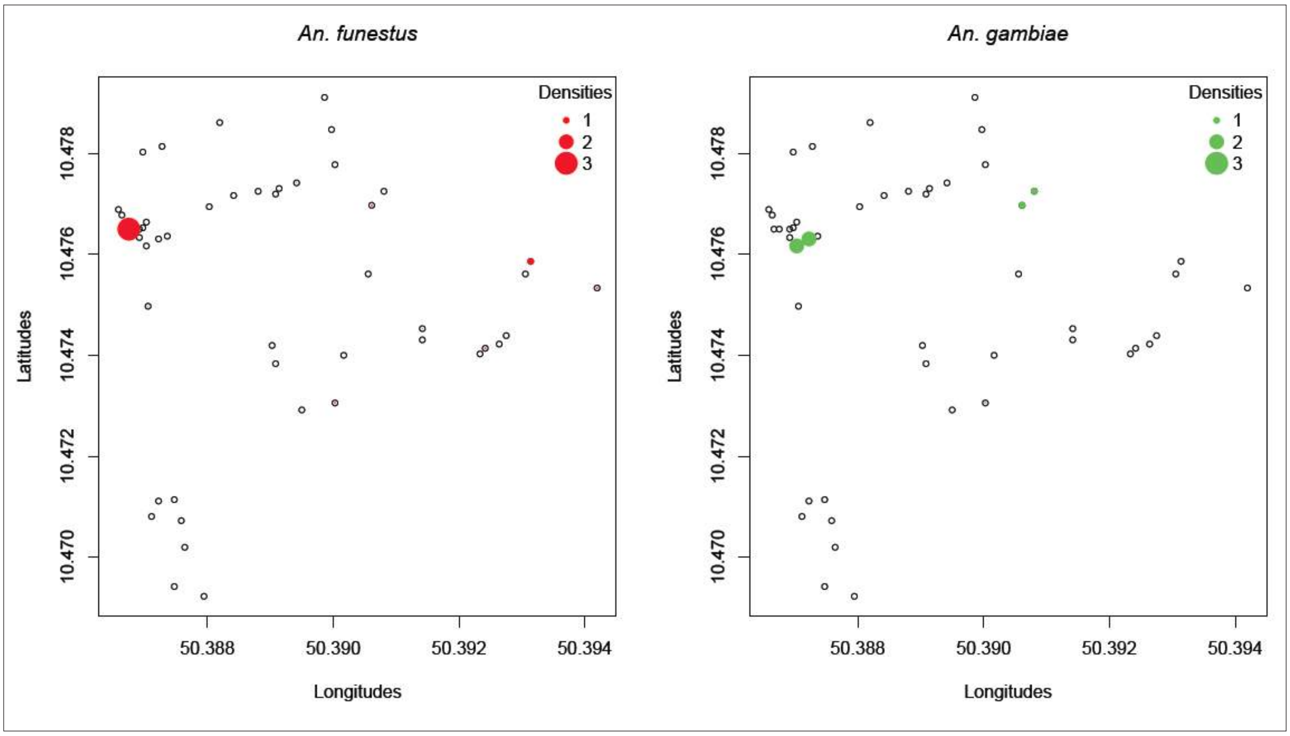
**Biyokulule**



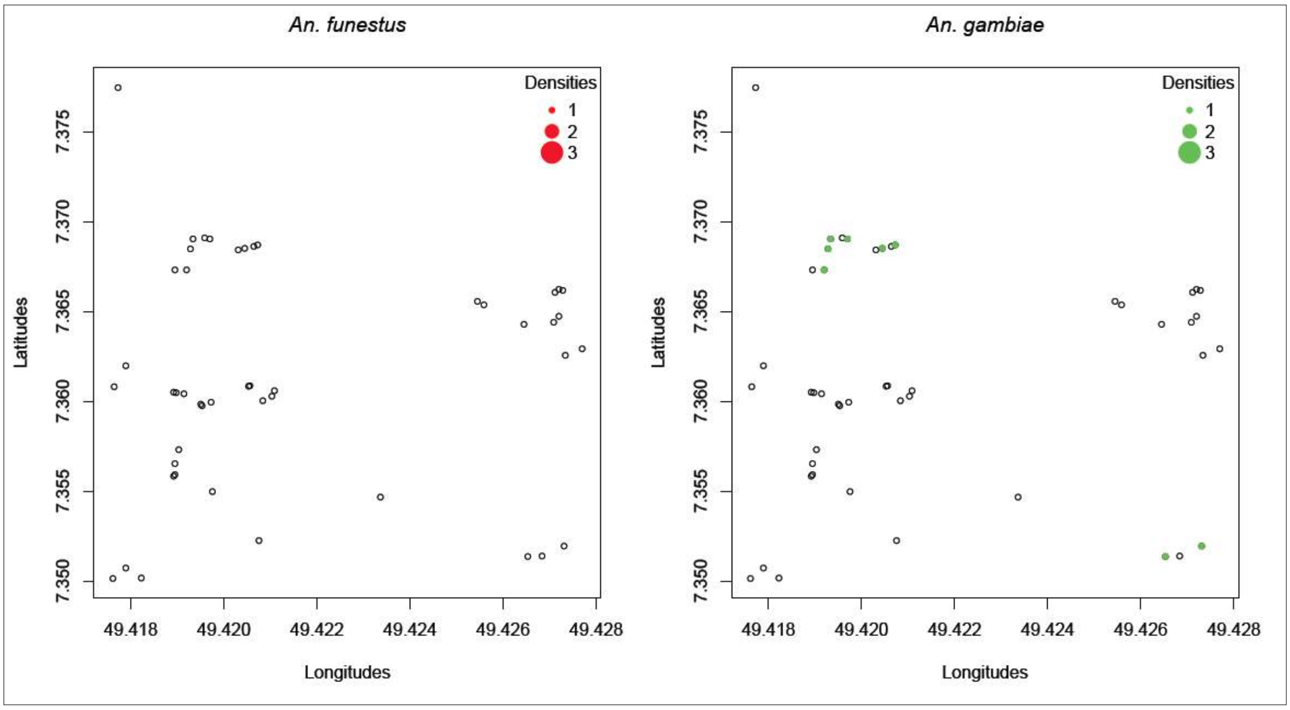
**Burtinle**




**Iskushuban**

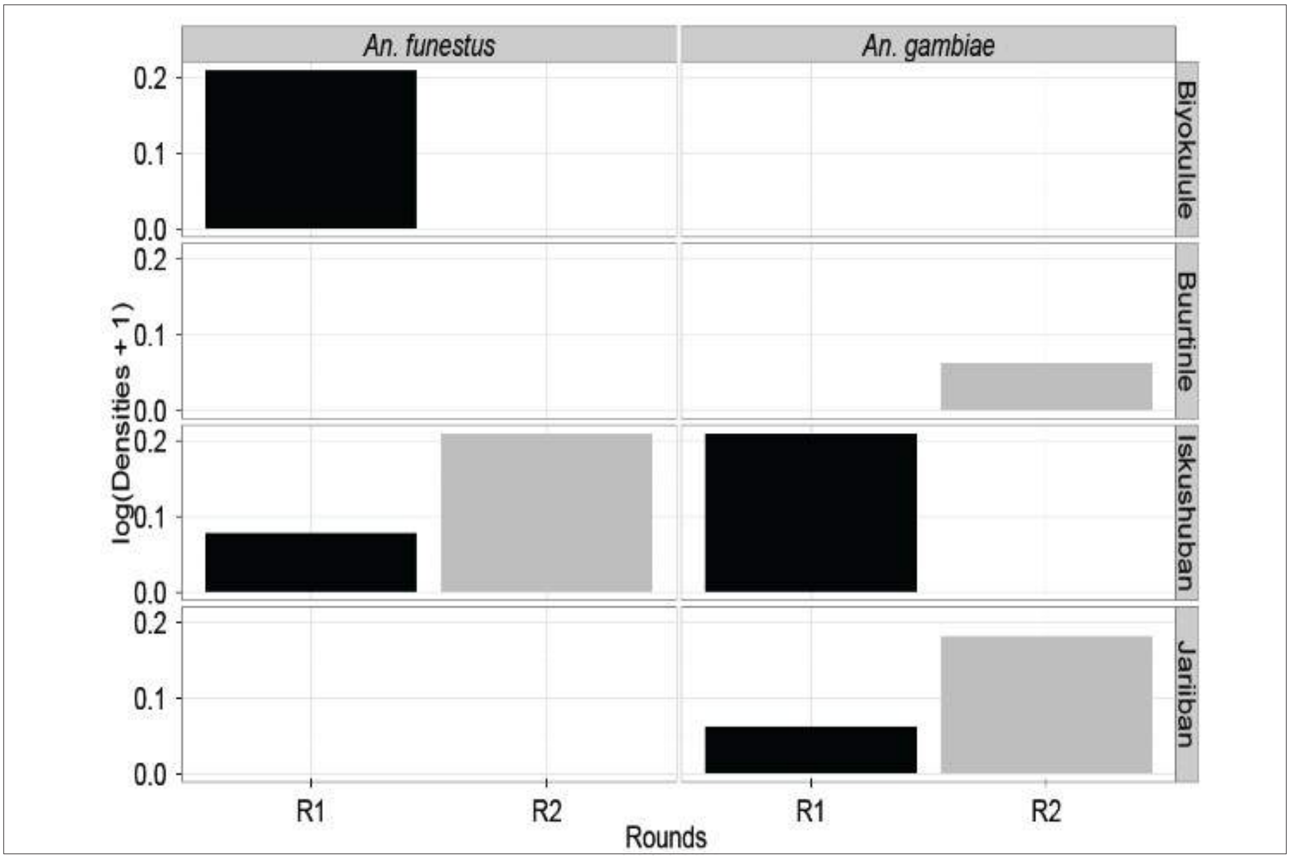


**Jeriiban**




Considering the round of collection, the first was globally more productive than the second (Figure 4). *An. funestus* resting densities was significantly higher during the first round in Biokulule (p-value = 0.005561). In Iskushuban, even though samples from the second round were higher, the difference with the first was not significant (p-value = 0.2785). For *An. gambiae* the first round was significantly higher than the second only in Iskushuban (p-value = 0.002749).

**Figure 4: Dynamic of *An. gambiae* and *An. funestus* according to round of sampling.**



The indoor resting behaviour has been compared according to the structure of the household (Table 5) and the application or not of Indoor residual spray (IRS) of Insecticides (Table 6). Cement and metallic shelters were the unique type of structure investigated. They represented respectively 85% and 25%. While the most important number of specimens have been collected in the habitations made on cement the vector densities were comparable in all villages for both *An. gambiae* (p-value = 0.6018) and *An. funestus* (0.1983 ≤ p-value ≤ 0.3348).

**Table 5 : Indoor resting densities for *An. gambiae* and *An. funestus* according to the structure of households investigated in Puntland**

| | Type | Number | *An. gambiae* | | *An. funestus* | |
|---|---|---|---|---|---|---|
| **Villages** | **Structure** | **Bedrooms** | **Number of specimens** | **F/br** | **Number of specimens** | **F/br** |
| Biyo kulule | Cement | 58 | 0 | 0 | 7 | 0.121 |
| | Metallic Shelter | 21 | 0 | 0 | 3 | 0.143 |
| Buurtinle | Cement | 71 | 2 | 0.028 | 0 | 0 |
| Iskushuban | Cement | 56 | 7 | 0.125 | 10 | 0.178 |
| | Metallic Shelter | 24 | 1 | 0.042 | 1 | 0.042 |
| Jariiban | Cement | 70 | 8 | 0.114 | 0 | 0 |
| **Total** | Cement | **255** | **10** | **0.039** | **17** | **0.067** |
| | Metallic Shelter | **45** | **1** | **0.022** | **4** | **0.089** |

**F/br : female per bedrooms.**

Concerning the influence IRS, only one village was submitted to its application. However the densities of *An. funestus* the unique malaria vector species recorded in both type of bedrooms did not revealed significant difference (p-value = 0.7222).

**Table 6 : Indoor resting densities for *An. gambiae* and *An. funestus* in households investigated in Puntland according to their treatment with insecticide**

| | IRS | | | | | No-IRS application | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Locality** | | ***An. gambiae*** | | ***An. funestus*** | | | ***An. gambiae*** | | ***An. funestus*** | |
| | **TBR** | **Nb** | **F/br** | **Nb** | **F/br** | **NTBR** | **Nb** | **F/br** | **Nb** | **F/br** |
| Biyo kulule | 49 | 0 | 0 | 4 | 0.082 | 30 | 0 | 0 | 6 | 0.2 |
| Buurtinle | 0 | 0 | 0 | 0 | 0 | 71 | 2 | 0.028 | 0 | 0 |
| Iskushuban | 0 | 0 | 0 | 0 | 0 | 80 | 8 | 0.1 | 11 | 0.137 |
| Jariiban | 0 | 0 | 0 | 0 | 0 | 70 | 8 | 0.114 | 0 | 0 |
| **Total** | **49** | **0** | **0** | **4** | **0.082** | **35** | **18** | **0.514** | **8** | **0.228** |

IRS : Indoor residual spray ; TRB : Treated bedrooms (bedrooms submitted to IRS) ; NTBR : Non treated bedrooms (No IRS application); Nb: Number of specimens collected

### *9.1.2 BITING BEHAVIOUR OF MALARIA VECTORS IN PUNTLAND*

A total of 412 Anopheles have been collected using the CDC_LT including *An. gambiae* (N=159) and *An. funestus* (N=253). The biting rate ranged from 0 - 5.12 mosquitoes per trap per night (M/T/N) for *An. gambiae* and 0 – 5.58 M/T/N for *An. funestus* (Table 7). The comparison of collection did not revealed for both species significant difference between the indoor and outdoor biting rates, in all location except in Buurtinle where *An. gambiae* exhibited a significant exophagous tendency (p-value = 0.004375).

**Table 7: Malaria vectors species collected using CDC_LT and density estimated as the number of female mosquitoes per trap per night.**

| | *An. gambiae* | | *An. funestus* | |
|---|---|---|---|---|
| Locality | **In** | **Out** | **In** | **Out** |
| Biyokulule | 0 | 0 | 4 (0.1) | 0 |
| Iskushuban | 5 (0.2) | 6 (0.25) | 134 (5.58) | 114 (4.75) |
| Buurtinle | 7 (0.29) | 0 | 0 | 0 |
| Jariiban | 123 (5.12) | 18 (0.75) | 0 | 1 (0.04) |
| Total | 135 (1.20) | 24 (0.21) | 138 (1.23) | 115 (1.03) |

( ) number of individual per CDC_LT per night.

*An gambiae* has shown to be significantly more endophagous than *An. funestus* in Buurtinle (p- value = 0.007107) and Jariiban (p-value = 0.01278). Iskushuban is the only location where *An. funestus* was significantly more endophagous that *An gambiae* (p-value = 0.02857). Concerning the outdoor biting behaviour no significant difference has been observed between the 2 malaria vectors.

While *An. gambiae* predominated the fauna in Jariiban, *An. funestus* was the most common species in Iskushuban (Table 8). Concerning *An. funestus* the biting rate was significantly higher in Iskushuban (OR= 3.40; CI95% [2.24-5.15]; p <0.00001) than other location while for *An. gambiae,* Jariiban recorded the highest densities (OR= 2.11 ; CI95% [1.44-3.10]; p = 0.0002).

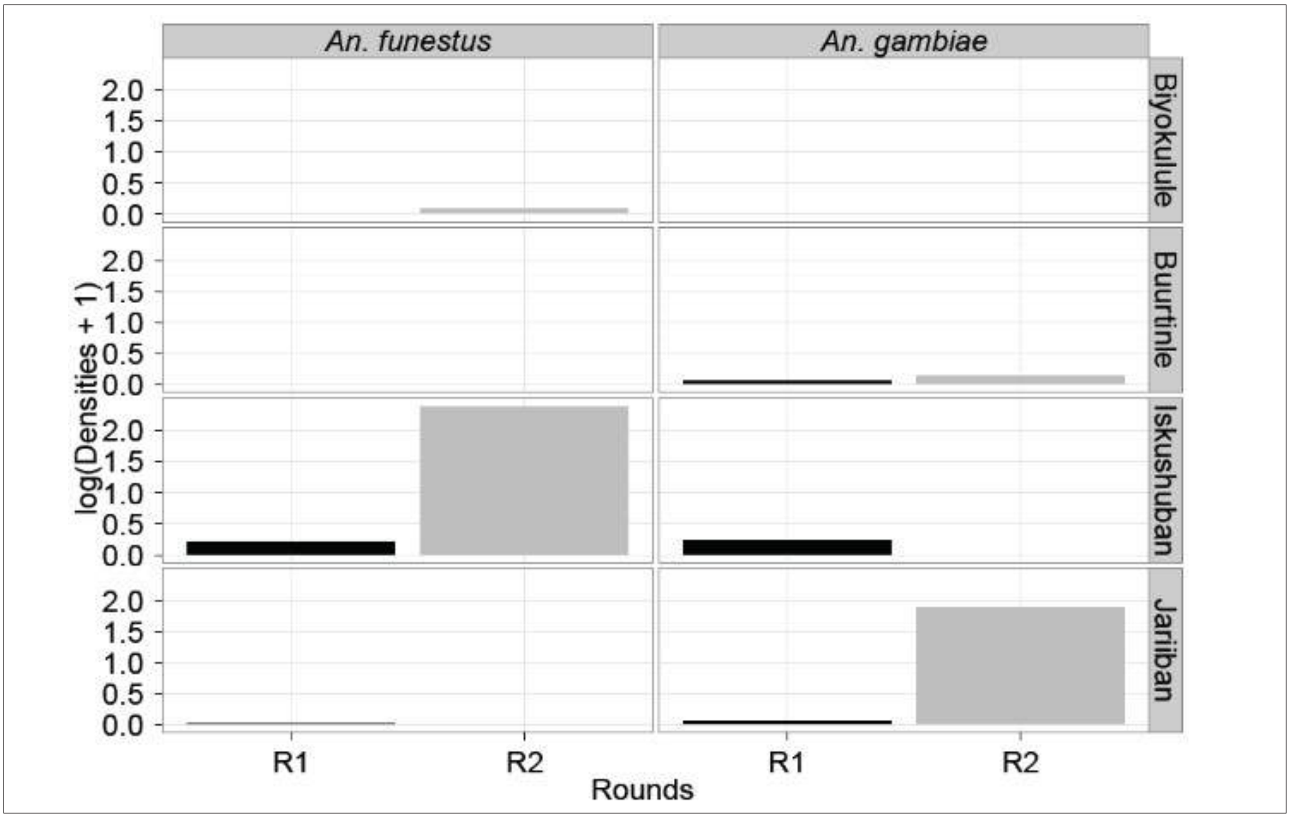
**Table 8 : Comparison of malaria vectors biting rate between villages**

| **Locality** | *An. funestus* | | | *An. gambiae s.l.* | | |
|---|---|---|---|---|---|---|
| | **OR** | **CI 95%** | **p** | **OR** | **CI 95%** | **p** |
| Biyokulule | 1 | | | 1 | | |
| Buurtinle | 0.94 | 0.62-1.43 | 0.79 | 1.11 | 0.75-1.62 | 0.60 |
| Iskushuban | 3.40 | 2.24-5.15 | <0.00001 | 1.10 | 0.75-1.62 | 0.61 |
| Jariiban | 0.96 | 0.63-1.46 | 0.860 | 2.11 | 1.44-3.10 | 0.0002 |

OR : Odds ratio ; CI : Confidence Interval

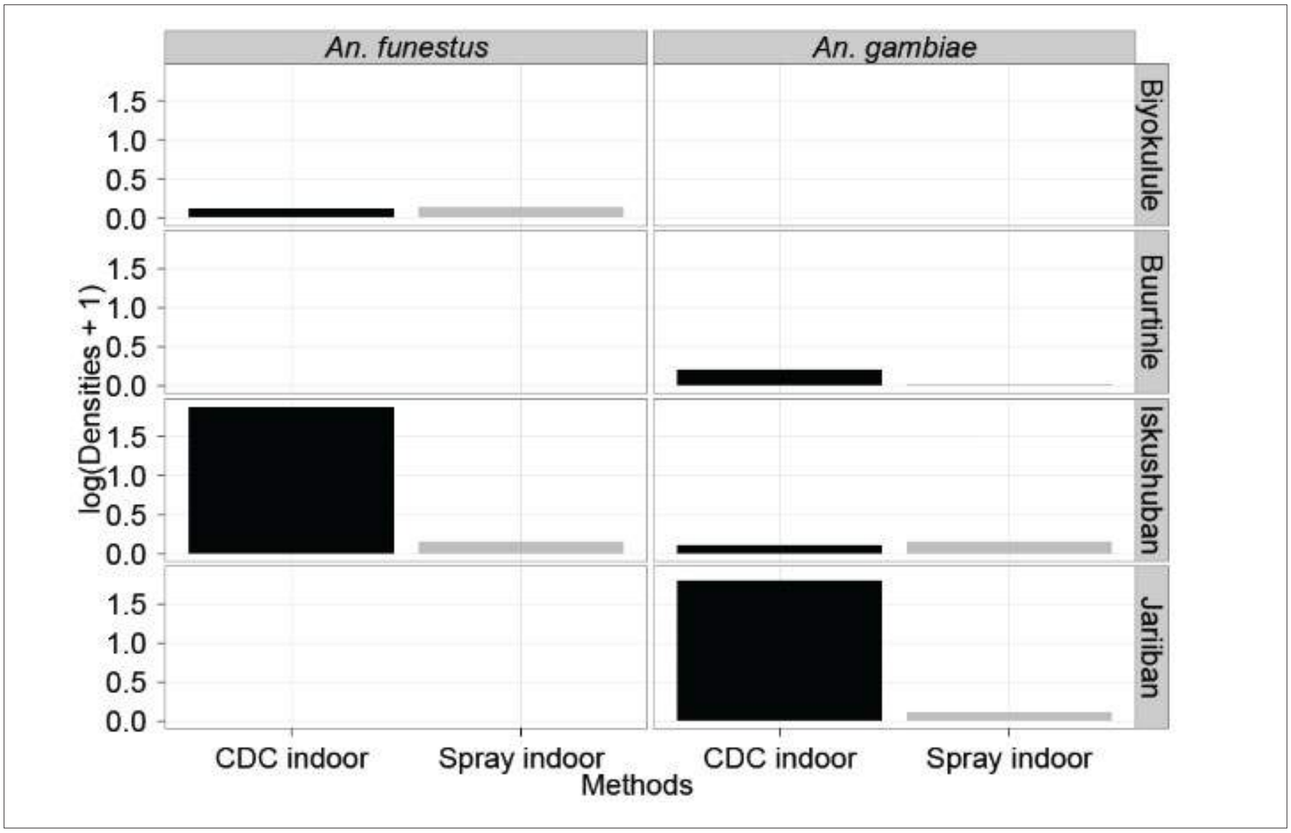
The figure 5 exhibits the variation of the densities of resting mosquito according to the round of collection in each location. Globally the densities were comparable except for *An. funestus* in Iskushuban (p-value = 0.0007781) and *An. gambiae* in Jariiban (p-value = 0.01587) exhibiting a significant increase of the density during the second round compared to the first round.

**Figure 5. Dynamic of mosquitoes biting rate according to round of collection**




## 9.1.3 COMPARISON INDOOR RESTING AND BITING RATE OF MALARIA VECTORS

**Figure 6. Comparison between indoor resting and biting rate of malaria vectors**



The outdoor resting behaviour of malaria vectors have been estimated in 7 houses of the selected villages using Window Exit method. The general observation is the weak number of mosquitoes collected by this method. Several hypothesis could explain this performance including i) an endophilic trend of the vector populations, ii) an inefficiency of the trap, iii) a non respect of consigns by the habitants of the houses where the traps were set up etc. However the data obtained from CDC-LT set up indoor are not consistent with the first hypothesis. Therefore to better appreciate the exophilic trends, face to the low performance of the window exit trap (WE), we have compared the indoor biting rate and the indoor residual resting densities (Figure 6) of the malaria vectors. The results have revealed a marked endophagic tendency of females, while the indoor resting densities were relatively low. This finding indicates that, after biting indoor, a large proportion of females get out the bedroom for resting. Such observation suggests an exophilic trend of the malaria vectors.

### *9.1.5 MALARIA VECTOR SPECIES IDENTIFICATION*

A total of 33 specimens of *An. gambiae s.l.* have been sent to Dakar for identification of members of the Anopheles gambiae complex including 15 from Buurtinle and 18 from Jariiban. All samples from Buurtinle have been identified as *An. arabiensis* while samples from Jariban included 15 *An. arabiensis* and 3 *An. gambiae s.s* further identified as the M molecular form currently called *An. coluzzii* (Coetzee et al., 2013).

Also 20 *An. funestus* from Iskushuban and 1 *An. rhodesiensis* have been confirmed morphologically.

### *9.1.6 FEEDING PATTERN OF THE MALARIA VECTORS*

All blood meal samples of the An. gambiae complex tested were from human (Table 9). Concerning An. funestus the majority of blood meal were from human also. The anthropophilic rate were 80% in Biyokulule and 92.8% in Iskushuban (Table 10). Also two mixed blood meal were recorded in Biyokulule including one taken on 2 different hosts (Ovine/chicken) and one on
3 different hosts (Human/Bovine/chicken).

**Table 9: Number and percentage of *An. arabiensis* and *An. gambiae s.s* females fed on each of the 5 common domestic vertebrate hosts tested**

| | ***An. arabiensis*** | | ***An. gambiae s.s* (M form/*An. coluzzii*)** | |
|---|---|---|---|---|
| **Locality** | **Number tested** | **Human** | **Number tested** | **Human** |
| Jariban | 10 | 10 (100) | 1 | 1 (100) |
| Buurtinle | 4 | 4 (100) | 0 | 0 |
| **Total** | **14** | **14 (100)** | **1** | **1 (100)** |

( ) : Percentage (%).

**Table 10: Number and percentage of *An. funestus* females fed on each of the 5 common domestic vertebrate hosts tested**

| **Locality** | **Number tested** | **Single blood meal** | | **Mixed blood meal** | |
|---|---|---|---|---|---|
| | | **Human** | **Bovine** | **Ovine/chicken** | **Human/Bovine/chicken** |
| Iskushuban | 14 | 13 (92.8) | 1 (7.1) | 0 | 0 |
| Biyokulule | 10 | 8 (80) | 0 | 1 (10) | 1 (10) |
| **Total** | **24** | **21 (87.5)** | **1 (4.1)** | **1 (4.1)** | **1 (4.1)** |

( ) : Percentage (%).

Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, et al. (2013) *Anopheles coluzzii* and *Anopheles amharicus*, new members of the Anopheles gambiae complex. Zootaxa 3: 246–274.

## 9.2 VECTORS BEHAVIOUR IN SOMALILAND

From Somaliland overall, 674 Anopheles, belonging to 11 species were collected (Table 11). Among the Anopheles fauna, *An. dthali* was the most abundant species (n=472; 70%), followed by *An. rhodesiensis* (n=72; 10.7%), *An. garnhami* (n=62; 9.2%), *An. turkhudi* (n=22; 3.3%) and *An. culicifascies* (n=17; 2.5%). Only 3 specimens of *An. gambiae s.l* have been collected in Biyooley.

Globally the data analysis using Kruskall-Wallis test, did not revealed significant difference between Round 1 vs Round 2 collections for *An. dthali* in Turka (chi-squared = 2.763, df = 1, p- value = 0.09646), Agabar (chi-squared = 0.9419, df = 1, p-value = 0.3318), Ali haid (chi-squared = 1.3728, df = 1, p-value = 0.2413) and Biyooleye (chi-squared = 1.0105, df = 1, p-value = 0.3148). The same trend was observed for other species except for *An. rhodesiensis* exhibiting a significant difference in Biyooleye between R1 vs R2 collections (chi-squared = 6.07, df = 1, p- value = 0.01375).

### *9.2.1 RESTING BEHAVIOUR OF THE COMMON ANOPHELES IN SOMALILAND*

The indoor resting densities of the most common Anopheles mosquitoes were relatively low in all localities investigated (Table 12). They ranged from 0 to 1.027 mosquito females per bedrooms. The highest density has been recorded with *An. dthali* and in Biyooley.

**Table 11: Mosquito species collected using the different sampling methods from February 28th 2014 to March 31st 2014, in Somaliland.**

| | Turka | | | | Agabar | | | | AliHeid | | | | Biyooley | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LT | | Spray | Total | LT | | Spray | Total | LT | | Spray | Total | LT | | Spray | WE | Total | Total | % |
| **Species** | **In** | **Out** | **In** | | **In** | **Out** | **In** | | **In** | **Out** | **In** | | **In** | **Out** | **In** | **Out** | | | |
| *An. austeni* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| *An. azaniae* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.3 |
| *An. culicifascies* | 0 | 3 | 13 | 16 | 0 | 0 | 0 | 0 | | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 17 | 2.5 |
| *An. dthali* | 3 | 4 | 42 | 49 | 3 | 20 | 1 | 24 | 7 | 25 | 1 | 33 | 99 | 165 | 76 | 26 | 366 | 472 | 70.0 |
| *An. gambiae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 3 | 0.4 |
| *An. garnhami* | 7 | 13 | 29 | 49 | 0 | 0 | 0 | 0 | 2 | 10 | 1 | 13 | 0 | 0 | 0 | 0 | 0 | 62 | 9.2 |
| *An. gibbinsi* | 0 | 0 | 11 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 1.6 |
| *An. longipalpis* | 0 | 0 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 11 | 1.6 |
| *An. rhodesiensis* | 0 | 0 | 7 | 7 | 3 | 13 | 0 | 16 | 0 | 0 | 0 | 0 | 8 | 16 | 25 | 0 | 49 | 72 | 10.6 |
| *An. seydeli* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| *An. turkhudi* | 1 | 0 | 7 | 8 | 1 | 4 | 0 | 5 | 0 | 0 | 0 | 0 | 4 | 1 | 4 | 0 | 9 | 22 | 3.2 |
| **Total** | **11** | **20** | **119** | **150** | **7** | **39** | **1** | **47** | **9** | **37** | **2** | **48** | **111** | **182** | **110** | **26** | **429** | **674** | 10 |

In : Indoor ; Out : Outdoor

**Table 12: Indoor resting densities for *An. dthali, An. garnhami, An. rhodesiensis* and *An. turkhudi* in different villages of Somaliland (February 28th 2014– March 31st 2014)**

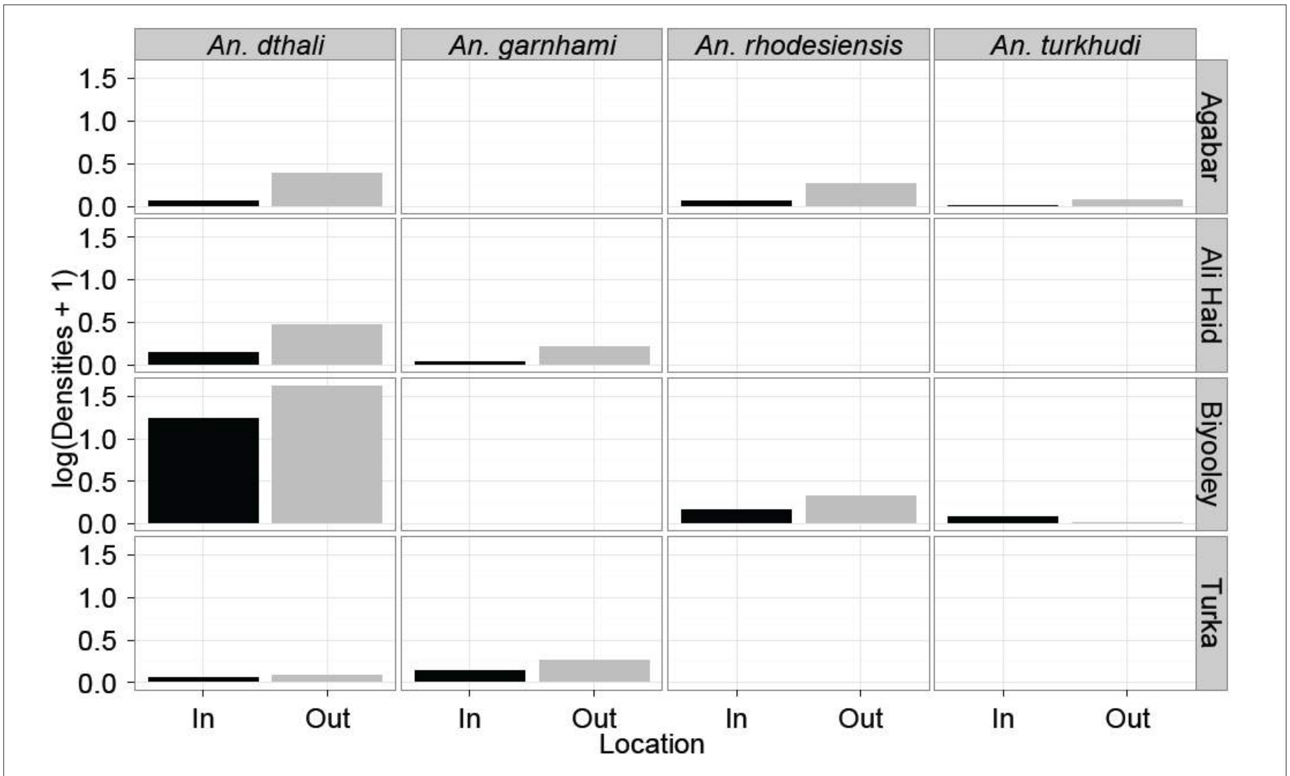
| | *An. dthali* | | | *An. garnhami* | | | *An. rhodesiensis* | | | *An. turkhudi* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Localities** | **n** | **br** | **f/br** | **n** | **br** | **f/br** | **n** | **br** | **f/br** | **n** | **br** | **f/br** |
| Agabar | 1 | 74 | 0.013 | 0 | 83 | 0 | 0 | 83 | 0 | 0 | 83 | 0 |
| Ali Heid | 1 | 83 | 0.012 | 1 | 83 | 0.012 | 0 | 83 | 0 | 0 | 83 | 0 |
| Biyooley | 76 | 74 | 1.027 | 0 | 74 | 0 | 25 | 74 | 0.337 | 4 | 74 | 0.054 |
| Turka | 42 | 91 | 0.461 | 29 | 91 | 0.318 | 7 | 91 | 0.077 | 7 | 91 | 0.077 |
| Total | 120 | 322 | 0.372 | 30 | 331 | 0.091 | 32 | 331 | 0.097 | 11 | 331 | 0.033 |

n= number of specimens : br = number of bedrooms ; f/br : number of females per bedroom

## 9.2.2 BITING BEHAVIOUR OF THE COMMON ANOPHELES IN SOMALILAND

The analysis of Figure 7 have showed that the biting rates were more important outdoor than indoor for the most common species, except in Biyooley where an opposite trend was observed with *An. Turkhudi*. However the differences observed is not statistically significant.

**Figure 7 : density of *An. dthali, An. garnhami, An. rhodesiensis, An. turkhudi* collected using CDC_LT and estimated as the number of female mosquitoes per trap per night**.




The biting rates of these common species were also comparable between the different villages except for *An. dthali* exhibiting a biting rate significant higher in Biyooley compared to other villages (Table 13)

**Table 13 : Comparison of *An. dthali, An. garnhami, An. rhodesiensis, An. tukhudi* biting rates between villages in Somaliland Villages**

| Species | | Agabar | Ali Heid | Biyooley | Turka |
|---|---|---|---|---|---|
| | 0R | 1 | 1.09 | 3.32 | 0.86 |
| *li* | CI 95% | | 0.60 – 1.97 | 1.83 – 6.03 | 0.47 – 1.55 |
| | P | | P=0.71 | P=0.005 | P=051 |
| | 0R | 1 | 1.14 | 1 | 1.25 |
| *An. garnhami* | CI 95% | | 0.93-141 | 0.81-1.23 | 1.01-154 |
| | P | | 0.14 | 1 | 0.04 |
| | 0R | 1 | 0.96 | 1.09 | 0.84 |
| *An. rhodesiensis* | CI 95% | | 0.71-1.30 | 0.80-1.48 | 062-1.14 |
| | P | | 0.73 | 0.5 | 0.18 |
| | 0R | 1 | 0.94 | 1 | 0.95 |
| *udi* | CI 95% | | 0.85-1.04 | 0.90-1.11 | 0.86-1.05 |

P 0.17 1 0.26

### 9.2.2 MALARIA VECTOR SPECIES IDENTIFICATION

A total of 17 *An. gambiae s.l* have been collected. All samples were identified as *An. arabiensis* by PCR. The species exhibited an endophilic tendency since no specimen has been collected using WET. They also exhibited an endophagic trend with 89% of samples collected indoor by CDC-LT.

**Table 14: Samples of Anopheles identified as *An arabiensis* by PCR**

| Locality | CDC-LT | | Spray | WET | Total |
|---|---|---|---|---|---|
| | Indoor | Outdoor | Indoor | Outdoor | |
| Agabar | 1 | 0 | 0 | 0 | **1** |
| Ali Heid | 0 | 1 | 0 | 0 | **1** |
| Biyoley | 0 | 0 | 1 | 0 | **1** |
| Turka | 7 | 0 | 7 | 0 | **14** |
| **Total** | **8** | **1** | **8** | **0** | **17** |

## 9.2 VECTORS BEHAVIOUR IN SOUTH CENTRAL ZONE (SCZ)

A total of 6135 mosquitoes have been collected from the 4 villages of the South Central zone with a large majority of Culicines (97.59%). The Anophelines counted globally for 2.56% (Table 15) in abundance varying according to the localities from 0 to 3 %. In total 11 species of Anopheles haves been recorded in the collection and 25.50% of the Anophelines have not been identified at the species level. (Table 16). Among Anophelines, *An. gambiae* (48.32%) was dominant followed by *An. funestus* (12.75%).

**Table 15 : Mosquitoes fauna collected using the different sampling methods from March 2014 to April 2014, in SCZ.**

| | Round 1 | | | Round 2 | | | Total | Total | |
|---|---|---|---|---|---|---|---|---|---|
| **Villages** | **Anopheles** | **Culicines** | **Total** | **Anopheles** | **Culicines** | **Total** | **Anopheles** | **Culicines** | **Total** |
| **Jowhar Somali** | 5 | 518 | 523 | 29 | 601 | 630 | 34 | 1119 | 1153 |
| **Jowhar Moyki** | 12 | 578 | 590 | 50 | 1474 | 1524 | 62 | 2052 | 2114 |
| **Jambaluul** | 2 | 177 | 179 | 26 | 728 | 754 | 28 | 905 | 933 |
| **Wanla-weyn** | 0 | 485 | 485 | | | | 0 | 485 | 485 |
| **Marerey** | | | | 37 | 1574 | 1601 | 37 | 1574 | 1601 |
| **Total** | 19 | 1758 | | 142 | 4377 | | 161 | 6135 | 6286 |
| | (1.07) | (98.93) | 1777 | (3.15) | (97.07) | 4509 | (2.56) | (97.59) | |

No sampling

**Table 16 : Anopheles species collected using the different sampling methods from March 2014 to April 2014, in South Central Zone**

| ***Species*** | **Jowhar Somali** | | | | **Jowhar Moyki** | | | | **Jambaluul** | | | | **Wanla-weyn** | | | | **Marerey** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CDC | Spray | WE | Total | CDC | Spray | WE | Total | CDC | Spray | WE | Total | CDC | Spray | WE | Total | CDC | Spray | WE | Total | **Total** | % |
| *An. gambiae* | 3 | 7 | 1 | 11 | 24 | 23 | 0 | 47 | 0 | 3 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 8 | 1 | 9 | **72** | **48.3** |
| *An. funestus* | 0 | 0 | 2 | 3 | 3 | 0 | 0 | 3 | 4 | 4 | 0 | 8 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 6 | **19** | **12.7** |
| *Anopheles sp* | 6 | 12 | 2 | 20 | 3 | 10 | 0 | 13 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **38** | **25.5** |
| ***An. implex*** | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1** | **0.6** |
| *An nili* | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1** | **0.6** |
| *An. azaniae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **3** | **2.0** |
| *An. daudi* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** | **1.3** |
| *An. pharoensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | **5** | **3.3** |
| *An. rhodesiensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | **4** | **2.6** |
| *An. coustani* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | **2** | **1.3** |
| *An. squamosus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1** | **0.6** |
| *An. dthali* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | **1** | **0.6** |
| **Total** | **9** | **21** | **5** | **35** | **30** | **33** | **0** | **63** | **7** | **19** | **2** | **23** | **0** | **0** | **0** | **0** | **4** | **18** | **1** | **23** | **149** | **100** |

CDC: CDC light Trap ; WE: Window Exit Trap

### 9.2.1 RESTING BEHAVIOUR OF MALARIA VECTORS IN SCZ

A total of 37 mosquitoes have been collected using the indoor pyrethrum spray catches (SP) in 828 bedrooms, (387 during the first round and 441 during the second round). Like other zone, the indoor resting density was also very low (Table 17). *An. gambiae* was significantly most abundant indoor than *An. funestus* (p-value = 0.0001049). The highest densities have been record in Jowhar Moyki for *An. gambiae* with globally 0.088 females per bedrooms. *An. funestus* have been collected only in Jambaluul and Marerey with respectively 0.032 and 0.023 f/br. However comparison using Krsuskal Wallis test did not revealed significant difference between the villages for *An. funestus* (chi-squared = 3.4466, df = 4, p-value = 0.486) as well as for *An. gambiae* (chi-squared = 6.543, df = 4, p-value = 0.1621).

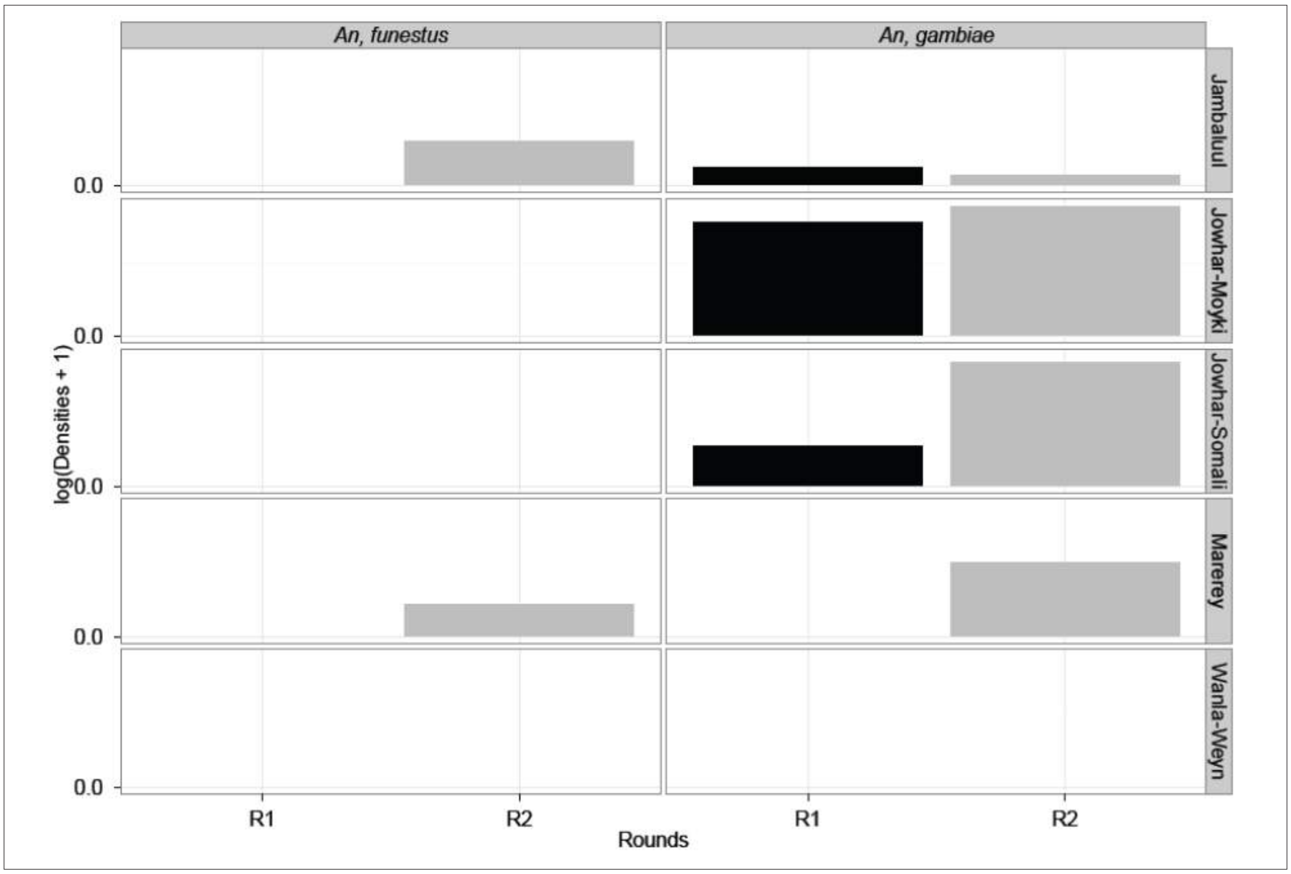
**Table 17 : Indoor resting densities for *An. gambiae* and *An. funestus* in different villages of SCZ**

| Villages | *An. gambiae* | | | *An. funestus* | | |
|---|---|---|---|---|---|---|
| | Number of specimens | Number of bedroom | Densities (f/br) | Number of specimens | Number of bedroom | Densities (f/br) |
| Jambaluul | 3 | 261 | 0.011 | 4 | 261 | 0.015 |
| Jowhar-Somali | 7 | 124 | 0.056 | 0 | 124 | 0 |
| Jowhar-Moyki | 20 | 228 | 0.088 | 0 | 228 | 0 |
| Wanla-Weyn | 0 | 83 | 0 | 0 | 83 | 0 |
| Marerey | 7 | 132 | 0.053 | 3 | 132 | 0.023 |
| **Total** | **37** | **828** | **0.045** | **7** | **828** | **0.008** |

f/br : females per bedroom

This vector density was variable according to round of collection. *An. funestus* was only recorded during the second round. The densities of *An. gambiae* were comparable between the 2 round of collection in all location. While in Jowhar Somali the second round seems to be more important, the difference was not significantly (p-value = = 0.7154).

**Figure 8 : Dynamic of *An. gambiae* and *An. funestus* according to round of**



The influence of the structure of the habitations on the resting behaviour has been analysed. In total 828 bedrooms have been investigated including 756 made on mud, 40 on stone and 32 metallic shelters (Table 18). Globally no significant different was observed between the densities of *An. gambiae* according to the type of structure using Krsuskal Wallis for *An. funestus* (chi-squared = 0.2087, df = 3, p-value = 0.9762) and for *An. gambiae* (chi-squared = 2.1837, df = 3, p-value = 0.5352). This trend has been observed in each village with *An. funestus* (p-value = 0.91) and for *An. gambiae* (0.34≤p-value ≤ 0.77).

**Table 18 : Indoor resting densities for *An. gambiae* and *An. funestus* according to the structure of households investigated in SCZ (March 2014 – April 2014)**

| | Type | | *An. gambiae* | | *An. funestus* | |
|---|---|---|---|---|---|---|
| **Villages** | **Structure** | **Bedrooms** | **Number of specimens** | **F/br** | **Number of specimens** | **F/br** |
| Jambaluul | Metallic Shelter | 32 | 0 | 0 | 0 | 0 |
| | Mud | 225 | 3 | 0.013 | 5 | 0.022 |
| | Stone | 4 | 0 | 0 | 0 | 0 |
| Jowhar-Somali | Mud | 110 | 6 | 0.54 | 0 | 0 |
| | Stone | 14 | 0 | 0 | 0 | 0 |
| Jowhar-Moyki | Mud | 206 | 20 | 0.097 | 0 | 0 |
| | Stone | 22 | 0 | 0 | 0 | 0 |
| Wanla-Weyn | Mud | 83 | 0 | 0 | 0 | 0 |
| Marerey | Mud | 132 | 7 | 0.053 | 2 | 0.022 |
| **Total** | **Metallic Shelter** | **32** | **0** | **0** | **0** | **0** |
| | **Mud** | **756** | **36** | **0.047** | **8** | **0.010** |
| | **Stone** | **40** | **0** | **0** | **0** | **0** |

Concerning the indoor residual spray (IRS) treatment, among the 828 bedrooms investigated 145 were submitted to IRS application, and 683 were not treated (Table 19). For *An. funestus* the indoor resting densities were significantly more important in bedrooms with IRS application than non- treated bedrooms (p-value = 0.001402) in Marerey. In Jambaluul while those non- treated recorded the highest densities the difference was not significant (p-value = 0.46). Concerning *An. gambiae* all specimens have been collected in untreated bedrooms. However the difference was not statistically significant (0.19 ≤p-value ≤ 0.6787).

**Table 19 : Indoor resting densities for *An. gambiae* and *An. funestus* in households investigated in SCZ according to their treatment with insecticide (March 2014– April 2014)**

| | IRS | | | | | No-IRS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *An. gambiae* | | | *An. funestus* | | *An. gambiae* | | | *An. funestus* | |
| | **TBR** | **Nb** | **F/br** | **Nb** | **f/br** | **NTBR** | **Nb** | **F/br** | **Nb** | **f/br** |
| Jambaluul | 99 | 0 | 0 | 0 | 0 | 162 | 3 | 0.018 | 5 | 0.031 |
| Jowhar-Somali | 9 | 0 | 0 | 0 | 0 | 115 | 6 | 0.052 | 0 | 0 |
| Jowhar-Moyki | 19 | 0 | 0 | 0 | 0 | 209 | 20 | 0.096 | 0 | 0 |
| Wanla-Weyn | 8 | 0 | 0 | 0 | 0 | 75 | 0 | 0 | 0 | 0 |
| Marerey | 10 | 0 | 0 | 3 | [0.3] | 122 | 7 | 0.057 | 0 | 0 |
| **Total** | **145** | **0** | **0** | **3** | **0.021** | **683** | **36** | **0.053** | **0** | **0** |

IRS : Indoor residual spray ; TRB : Treated bedrooms (bedrooms submitted to IRS) ; NTBR : Non treated bedrooms (No IRS application)

### 9.2.2 MALARIA VECTOR SPECIES IDENTIFICATION

To confirm the species identification some samples have been sent to Dakar. But the following issues have been recorded:

1. All the specimens were in sandwich between the desiccant (siliagel powder or granule formulation) and the cotton. Therefore most of them, mainly those stored in the granule formulation were destroyed.
2. Samples were received without their ID number. Only the name of the species was recorded on the vials introducing a major shortcoming for further data analysis in term of affectation to a geographic zone, house or method of collection.

The following results have been recorded :

- *An. arabiensis* has been the only species of the An. gambiae complex identified by PCR.
- From Marerey: 19 *An. arabiensis*; 3 *An. pharoensis*, 1 *An. rhodesiensis*, 3 *An. dthali*; 1
- Culicine sp (unidentifiable)
- From Jowhar Moyki : 44 *An. arabiensis*; 3 *An pharoensis*; 1 *An. funestus*.
- From Jambaluul: 9 *An. arabiensis*; 1 *An. pharoensis*; 2 empty vials ; 13 specimens unidentifiable completely destroyed.
- From Jowhar Somali: 25 *An. arabiensis*; 1 *An. funestus*; 2 *An. pharoensis*; 1 unidentifiable (recorded by the local team as *An. nili*).

### 9.2.3 FEEDING PATTERN OF THE MALARIA VECTORS

The majority of blood meals were of human (93.8%) origin in the SCZ (Table 20). The anthropohilic rate ranged from 80 in Jambaluul to 100% in Jowhar Somali. Only one patent mixed blood meal was taken on two different hosts (Human and chicken) in Marerey Also only one blood meal from Jambaluul did not react with the main domestic vertebrates antibodies tested.

**Table 20: Number and percentage of *An. arabiensis* females fed on each of the 5 common domestic vertebrate hosts tested**

| Locality | Single blood meal | | | | | | Mixed blood meal | |
|---|---|---|---|---|---|---|---|---|
| | Bovine | Ovine | Chicken | Equine | Human/Chicken | | Un-determined | |
| Jowhar Moyki | 17 | 16 (94.1) | 1 (5.8) | 0 | 0 | 0 | 0 | 0 |
| Jowhar Somali | 14 | 14 (100) | 0 | 0 | 0 | 0 | 0 | 0 |
| Jambaluul | 5 | 4 (80) | 0 | 0 | 0 | 0 | 0 | 1 (20) |
| Marerey | 13 | 12 (92.3) | 0 | 0 | 0 | 0 | 1 (7.7) | 0 |
| **Total** | **49** | **46 (93.8)** | **1 (2)** | **0** | **0** | **0** | **1 (2)** | **1 (2)** |

( ) : Percentage (%).

# 10.0 DISCUSSION

Two (2) rounds of entomological survey have been carried out in 4 villages selected in each of the three Somalia zones from February 28th 2014 to April 2014 with the main objective to investigate the resting and biting behaviour of *An. gambiae* complex and *An. funestus* and other potential malaria vectors and recommend appropriate vector control strategies. The indoor and outdoor mosquito resting behaviour have been estimated in each village using spray catch method and Window Exit trap. Also the indoor and outdoor biting behaviour of the malaria vectors have been estimated using 10 CDC Light Trap (CDC_LT) set up in rooms and courtyards of houses near the sleeper protected by an untreated bed net. Except the window exit trap the other collection methods used (CDC_LT and Spray) were enough efficient.

The data collected revealed that An. funestus (n=276) and An. gambiae (n=178) are the most common malaria vectors in Puntland with highest activities recorded in Iskushuban and Jariiban. For all vectors, a marked endophagic tendency was observed, while the indoor resting densities were relatively low indicating that, after biting indoor, a large proportion of females get out the bedroom for resting. No impact of the structures has been observed. The IRS application have been recorded in only one village but without impact on the malaria vector collected.

In Somaliland, only 17 specimens of *An. gambiae* have been recorded among 674 specimens of Anopheles (11 species) collected. *An. dthali* (n=472), *An. rhodesiensis* (n=72), *An.garnhami* (n=62) were dominant with also a low indoor resting densities. The low density of *An. gambiae* and the absence of *An. funestus* could be explained by a specific population dynamic uncompatible with the period of study or the a simple rarety of the species in the zone. In this later hypothesis other malaria vectors should be underconsideration.

In the SCZ, 6135 mosquitoes have been collected including 97.59% of Culicines and 2.56% of Anophelines. Among 11 species of Anophelines identified, *An. gambiae* and *An. funestus* represented respectively 48.32% and 12.75%. Like other zones, the indoor resting density was also very low. Except for Marerey exhibiting an indoor resting density for *An. funestus* significantly high in insecticide treated bedroom compared to untreated bedroom, in other villages the malaria vectors were only recorded in untreated bedroom. Concerning the structure of habitation no influence of have been recorded.

*An. arabiensis* was the unique or most common species recorded after confirmation by PCR in localities where the specimens of the complex have been collected. Only 3 specimens of An. gambiae ss belonging to the molecular form M has been recorded in Jariban (Puntland zone).

Blood fed specimens from Puntland and South Central Zone tested, have revealed that the majority of blood meals were of human origin. The anthropophilic rate was of 87.5 for *An. funestus* and ranged 93.8% - 100% for *An. gambiae*.

# 11.0 RECOMMENDATIONS

In view of the findings described above the following actions should be under consideration

- To Conduct a longitudinal study over a period of one year and at least in a monthly base to accurately estimate the vectors biodiversity and their seasonal variation. In fact, due to transversal nature of this present study, it is difficult to conclude about the list and prevalence of malaria vectors in each site. Adverse environmental conditions or a temporal dynamics of vectors that did not reached their peak of activity during the investigation could explain the scarcity or absence of certain species in some sites.
- To study the key parameters involved in the estimation of malaria transmission (entomological inoculation rates – EIR, feeding pattern, longevity etc.) or vectorial capacity (VC). In our knowledge there is no data available in the three zone concerning the association between plamodium parasite and the mosquito species. This gap should be filled.
- In area like Somaliand the role of the most common Anopheles in malaria transmission must be investigated. Such study could inlcude the mosquitoes biological parameters but also their potential to be infected by the malaria parasite.
- Data generated point out the importance to promote an integrated vector control approaches. High densities of resting Anopheles populations in the different areas would recommend using the IRS as the primary technique for vector control. However, alone it could not be enough regarding the proportion of vector biting or resting outdoor. Indeed in some places results have revealed a marked endophagic tendency of females, while the indoor resting densities were relatively low indicating that, after biting indoor, a large proportion of females get out the bedroom for resting.

## APPENDIX SUMMARY TABLE FOR BEHAVIOURAL STUDY

| Zone | Species | Resting | Biting location | Human biting rate | Feeding behaviour |
|---|---|---|---|---|---|
| Puntland | *An. arabiensis*<br>*An. coluzii*<br>*An. funestus* | High indoor resting densities of *Anopheles gambiae* in Jariban and Iskushuban. High indoor resting densities of Biyokulule and Iskushuban of *Anophesles funestus*. Overall higher indoor resting densities of *Anopheles gambiae* (small difference though) | *Anopheles gambiae* exhibited more endophilic and endophagic tendencies in Jariban. *Anopheles funestus* exhibited endophilic and endophagic tendencies in Iskushuban. No significant difference between in and outdoor collections - except in Burtinele, *Anopheles gambiae* tended to be more endophagic but had low endophilic endencies. Greater disparity between in and outdoor for An. *Gambiae* but less difference for *An. funestus* – this needs to investigated for a longer period of time. | Observation: There are differences of the biting behaviour by species in different villages: Jarriban : *Anopheles gambiae* displays more endophagic tendencies , while for *Anopheles funestus* displays more more endophagic tendencies in Iskushuban | *An. arabiensis*<br>*An. coluzii* – both anthrophophilic |
| Somaliland | *An. arabiensis* | Overall in Somaliland: highest indoor resting density in descending order: A. dthali>An. rhodensis>An. Gambiae | A lengthier study is needed before we can conclude malaria vectors are more outdoor biting than indoor – data was mostly taken from one village biyooley | *An. Garnhami* demonstrates more endophilic and endophagic tendencies in Turka. Anopheles dthali displays more endophilic and endopahgic tendencies in Biyooley. The decimal point is missing for Turka (table 13) – if it is Significant than An. Dthali demonstrates endophilic and endophagic tendencies. | **No data** |
| South Central | *An. arabiensis*<br>*An. pharoensis*<br>*An. funestus.* | Jowhar Moyki had the highest density *of An. arabiensis*, Maerey had the highest density of *Anopheles funestus* | **No data** | **No data** | An. arabiensis Anthrophophilic But need to look at the feeding preferences for An. funestus |