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Comparison of sampling techniques for Rift Valley Fever virus potential vectors, *Aedes aegypti* and *Culex pipiens* complex, in Ngorongoro District in northern Tanzania

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Abstract: We investigated mosquito sampling techniques with two types of traps and attractants at different time for trapping potential vectors for Rift Valley Fever virus. The study was conducted in six villages in Ngorongoro district in Tanzania from September to October 2012. A total of 1814 mosquitoes were collected, of which 738 were collected by CDC light traps and 1076 by Mosquito Magnet trapping technique. Of the collected mosquitoes, 12.46% (N= 226) were *Aedes aegypti* and 87.54% (N= 1588) were *Culex pipiens* complex. More mosquitoes were collected outdoors using Mosquito Magnets baited with octenol attractant, 36.38% (N=660) followed by indoor trapping using CDC light traps without attractant, 29.60% (N=537). Most of *Ae. aegypti* mosquitoes were collected outdoor using Mosquito Magnets, 95% (N=214) whereas *Cx. pipiens* complex were trapped both indoor using CDC light traps without attractant and outdoors using both CDC light traps baited with carbon dioxide (CO_2) sachets and Mosquito Magnets. Analysis on the differences in abundance of mosquitoes trapped by different techniques using Generalized Linear Models was statistically significance at p-value < 0.05 for both species. Three hours mosquito collections show differing patterns in activity, most *Ae. aegypti* species were collected primarily during the first and last quarters of the day. *Cx pipiens* complex was active throughout the night, early evening and early morning then decreased markedly during the day time. The results presented in this paper emphasize the possibility of using Mosquito Magnets in order to efficiently capture these potential RVF vectors.

Keyword: Rift Valley Fever, *Aedes aegypti*, *Culex pipiens*, mosquito sampling, trap, Tanzania

Introduction

Rift Valley fever (RVF) is a mosquito-borne arboviral infection caused by a virus belonging to the Phlebovirus genus of the Bunyaviridae family (Daubney *et al.*, 1931; Davies, 1975). The virus is passed from one generation of Aedes mosquito to another trans-ovarially (Logan *et al.*, 1991; Diallo *et al.*, 2000; Romoser *et al.*, 2011). This accounts for the continued presence of the RVF virus in enzootic foci and provides the virus with a sustainable mechanism of existence as eggs which can survive for several years in dry conditions (Logan *et al.*, 1991; Linthicum *et al.*, 1999; Gerdes, 2002; Nguku *et al.*, 2010). Known important RVF vectors in East Africa include *Aedes mcintoshi, Ae. ochraeus, Ae. dalzieli* and *Ae. vexans* (Logan *et al.*, 1990; Turell *et al.*, 2008; Sang *et al.*, 2010).

Aedes aegypti has been found naturally infected with RVF virus in Sudan in 2007 and has the ability to transmit the virus both mechanically and biologically (EFSA, 2013). Laboratory established colonies of *Ae. aegypti* from Tahiti exhibited the highest disseminated infection rates of RVF virus when compared with other potential vectors in the Mediterranean (Moutailler *et al.*, 2008). *Ae. aegypti* has also demonstrated infection and transmission rates of the non-structural NSs protein deletion virus similar to wild type virus while dissemination rates were significantly reduced (Crabtree *et al.*, 2012). *Culex pipiens* has been incriminated as the main RVF vector in Egypt (Meegan

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et al., 1980). Moreover, records have shown that populations of *Cx. pipiens* from the Maghreb are efficient experimental vectors of RVF (Amraoui *et al.*, 2012) and in South Africa (Jupp & Cornel, 1988). Despite a number of studies and records on RVF in Tanzania (Jost *et al.*, 2010; Mohamed *et al.*, 2010; Heinrich *et al.*, 2012), there is inadequate information on mosquitoes incriminated as principal vectors for RVF virus persistence and transmission.

Lack of reliable techniques for sampling RVF vectors to determine abundance and distribution pattern associated with the disease epidemics could be among the factors hindering availability of information on RVF vectors in Tanzania. Studies in Kenya have identified important techniques for sampling RVF vectors (Tchouassi *et al.*, 2012). Attempts to use such sampling techniques have been found to be useful in mosquito vectors for malaria and filariasis (Mboera *et al.*, 2000a,b; Korgaonkar *et al.*, 2008; Kitau *et al.,* 2010). Inadequate sampling of potential RVF vectors may lead to under-reporting the role of mosquito in transmission and persistence of RVF virus during inter-epidemics period. The objective of this study was to compare sampling techniques for potential mosquito vectors of Rift Valley Fever virus in northern Tanzania.

Materials and Methods

Study area

The study was conducted from September to October 2012 in Ngorongoro district Tanzania (Figure 1). Ngorongoro District (2°S45'50.4", 35°E34'04.8") is located in Arusha Region of northern Tanzania. According to the 2012 Tanzania National population and housing census, the population of the district was 174,278 (NBS, 2013). The district is considered as part of the Serengeti-Mara Ecosystem, which is defined by the limits of the annual wildlife migration. The district represents unique interaction between livestock, wildlife and human. Six villages, namely, Orgosorok, Soitsambu, Digodigo, Malambo, Sale and Pinyinyi were selected for the study.

Mosquito collection

Adult mosquito collections were made both outdoors and indoors. Indoor collections were made using CDC light traps. Un-baited light traps were set in four randomly selected houses in each village. Light traps were hung beside a sleeper who was provided with an untreated net as described by (Mboera *et al.*, 1998). A person in the house was instructed on how to how to set and retrieve the trap at 17:00hr and 07:00hr, respectively.

Outdoor mosquito collections were made using CDC light traps baited with carbon dioxide (CO_2) sachets and Mosquito Magnets (Cordless Liberty-Plus) alone or baited with Octenol. A Mosquito Magnet trap produces a continuous and odourless stream of CO_2, warmth and moisture into the air (Kline, 2006; Kitau *et al.*, 2010; Xue *et al.*, 2010). Traps were set in proximity to potential breeding sites and under canopy in banana plantations and in proximity to animals sleeping areas. After every three hours, inspection was done on each trap to recover any trapped mosquito. Traps were set repeatedly in each area for three consecutive days and nights during the study period. All mosquitoes collected were sorted according to site of collection, type of trap, time of collection. Mosquito species were identified morphologically using specific keys (Huang, 2001). Each trap site was geo-referenced.

Data analysis

Data was entered into Microsoft Excel 2010 for basic descriptive statistics using pivot tables and then imported for analysis using the IBM Statistical Package for Social Sciences (SPSS) Version 19 and R statistical package. Mosquito were categorized by species, trapping techniques and time for

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collection. Comparison between the total numbers of mosquitoes caught per trapping technique was carried out by considering the differences in abundance and species composition. In order to obtain more realistic non-negative values for this non-normally distributed observations, this analysis was done using Poisson Generalized Linear Models (with a log link) at significance level of p-value < 0.05 (Demetrio, 2012).

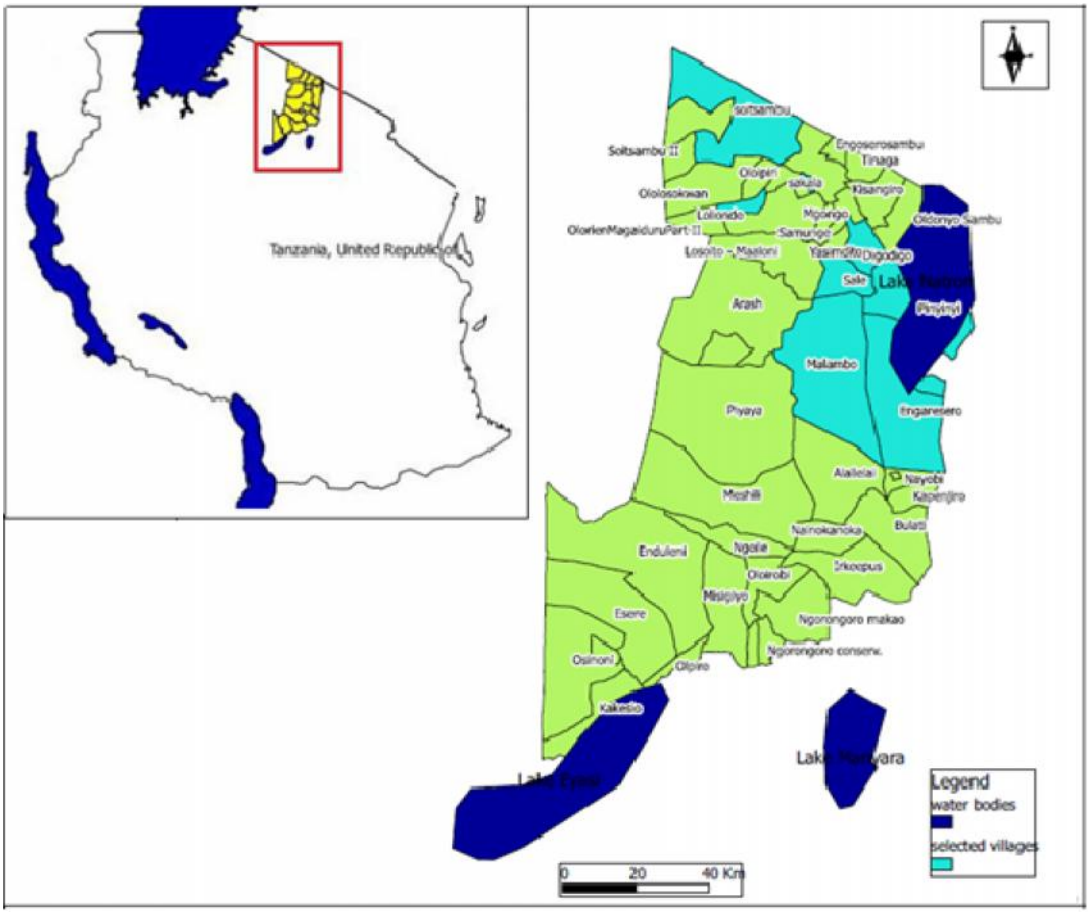

Figure 1: Map of Ngorongoro district indicating villages selected for the study

Ethical considerations

Ethical approval was sought from the Medical Research Coordinating Committee of the National Institute for Medical Research. Village leaders and house residents were asked for their consent before setting of mosquito traps in their houses or premises.

Results

Mosquito abundance and trap efficiency

A total of 1814 mosquitoes were collected, of which 40.68% (N= 738) were collected by CDC light traps and 59.32% (N= 1076) by Mosquito Magnet trapping technique. Of the collected mosquitoes, 12.46% (N= 226) were *Aedes aegypti* and 87.54% (N= 1588) *Culex pipiens* complex. More mosquitoes (N=660) were collected outdoors using octenol-baited Mosquito Magnets than indoor by un-baited CDC light traps (N= 537). Most of the *Ae. aegypti* mosquitoes were collected outdoor using Mosquito

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Magnets, 95% (N= 214) whereas *Cx. pipiens* complex were trapped both indoor using CDC light traps without attractant and outdoors using both CDC light traps baited with CO_2 and Mosquito Magnet traps (Figure 2). Most *Ae. aegypti* mosquitoes were collected in Digodigo (60.2%, N= 136) and Pinyinyi (23.9%, N= 54) whereas more *Cx. pipiens* were collected in Digodigo (41.7%, N= 667).

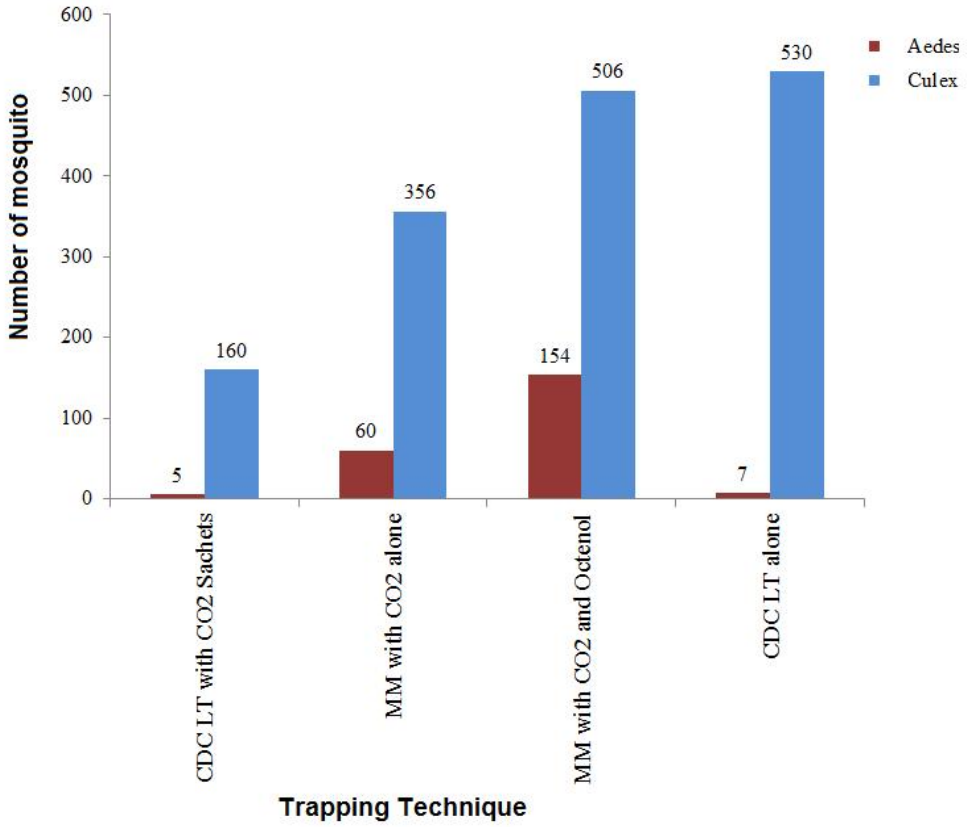

Figure 2: Number of *Aedes aegypti* and *Culex pipiens* mosquitoes trapped using Light traps (LT) and Mosquito Magnet (MM) traps

CDC light traps set indoor showed a low efficiency in catching *Ae. aegypti* with only seven mosquitoes captured indoors and five outdoors despite addition of CO_2 as attractant. In contrast, outdoor trapping using Mosquito Magnets alone or baited with Octenol captured the largest proportion of *Cx. pipiens* and *Ae. aegypti* (Figure 2). Most *Ae. aegypti* mosquitoes were captured in traps set under canopy in banana plantations and in proximity to animals sleeping area. In contrast, the number of *Cx. pipiens* trapped was similarly distributed both outdoors and indoors.

There were statistically significant differences in mosquito collection between different trapping methods. The indoor un-baited CDC light trap was more efficient in capturing *Cx. pipiens* (p-value <0.05). On the other hand, octenol-baited Mosquito Magnet set outdoor was more efficient to in collecting *Ae. aegypti* (p-value < 0.05). In this case both probabilities suggest strong evidence against the type of trap and attractant on the attractiveness to abundance of mosquito species.

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Mosquito activity patterns

Most *Ae. aegypti* mosquitoes were collected primarily during the first and last quarters of the day. This indicates activity of *Ae. aegypti* being a bimodal with one well-pronounced peak in the first three hours on the night (dawn) and another peak in the afternoon. Between these two peaks, mosquito activity was very low. *Cx pipiens* complex showed to be actively stable throughout the night, early evening and early morning then decreased markedly during the day time. In general, *Cx pipiens* showed a more pronounced nocturnal activity than *Ae. Aegypti* (Figure 3).

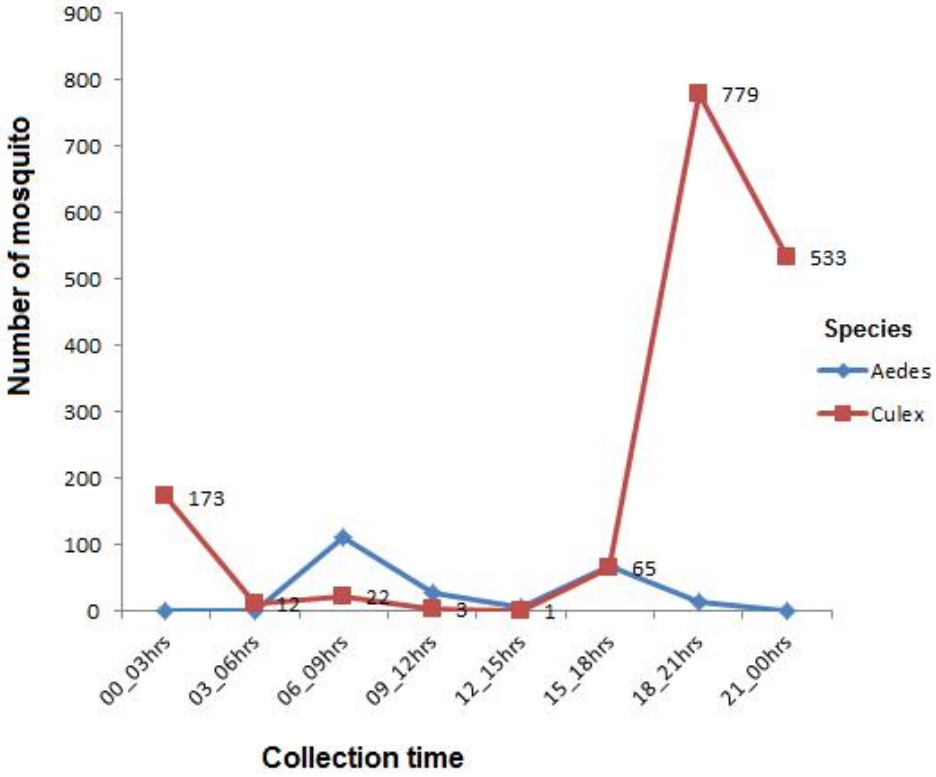

Figure 3: Mosquito activity patterns based on mosquito collection time

Discussion

Efficient sampling of disease vectors provide important information on disease transmission and can provide more understanding on effective vectors for targeted control strategies (Krockel *et al.*, 2006). Several mosquito sampling techniques are available have been mainly used to sample malaria and lymphatic filariasis vectors (Mboera et al., 1998; Mboera, 2005). The variation in mosquito activities observed in this study indicates that sampling of potential RVF vectors requires techniques that can maximize number of catches of both indoor and outdoor biting mosquitoes. *Cx. pipiens* complex indicated a similar activity pattern of being highly active during the evening to mid night with less or no activity during the day. This phenomenon has been observed in North Italy (Veronesi *et al.*, 2012). The bimodal activity with peaks at dawn and afternoon, observed among *Ae. aegypti* in this study was similar to observations on *Ae. albopictus* in Macao, China. Similar results have been observed in the La Reunion Island (Delatte *et al.*, 2010). This difference in catch between species, location and time provides important information for strategic vector sampling technique.

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The need for effective techniques for sampling potential RVF vectors is important as the role of mosquitoes in the maintenance and transmission of RVF virus in Tanzania is still not clear. Studies based on artificial flooding of grassland depressions in Kenya had led to identification of *Ae. mcintoshi, Ae. ochraeus, Ae. dalzieli* and *Ae. vexans* (Logan *et al.*, 1990; Turell *et al.,* 2008; Sang *et al.*, 2010) as important vectors of RVF virus in the area. *Ae. aegypti* and *Cx. pipiens* complex have been observed in several RVF epidemic zones but their role has not been confirmed. However, the two species have been found to play a significant role in other epidemic zones such as Egypt (Meegan *et al.*, 1980). Findings of this study have indicated that Mosquito Magnet trap is an efficient and practical tool for collecting *Ae. aegypti*. These traps have the advantage of collecting day biting outdoor mosquitoes because can easily be set and left in proximity with potential mosquito breeding areas.

In conclusion, because *Ae. aegypti* and *Culex pipiens* complex are important potential vectors of RVF, adequate sampling remain crucial in understanding of disease transmission during inter-epidemic periods. The results emphasize the possibility of using Mosquito Magnet traps in monitoring vector population dynamics in outdoor settings as are the best sampling tool for *Ae. aegypti*.

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Conflict of interest

The authors declare no conflicts of interest

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