STRENGTHENING THE CONTROL OF MOSQUITO VECTORS IN CABO VERDE; NEW APROACHES TO IMPROVE INTERVENTION STRATEGIES

Cabo Verde is an insular country located in the Atlantic Ocean and plagued by vector-borne diseases since its origins, with malaria outbreaks registered since the 16th century, with epidemics of yellow fever and lymphatic filariasis in the past and currently with large outbreaks of emerging viruses such as dengue and Zika. To respond to this priority health problem, the country has an integrated national program to combat vector-borne diseases, based on the use of insecticides (temephos for the control of breeding sites and deltamethrin for indoor and outdoor spraying). These control activities include the use of larvivorous fish, physical control of solid waste cleaning and communication campaigns during outbreaks and periods of greater risk of epidemics. Despite the efforts made, the epidemics of malaria and arboviruses (dengue and Zika) continue to be a reality. Partly due to failures in the design and development of the control program itself, but also due to the weather and the geopolitical position that Cape Verde plays, in the middle of the Atlantic between three continents, affected by the main traffic routes of trade and people.

To help the health authorities implement more assertive vector control strategies, the Tropical Diseases Research Group at the Jean Piaget University of Cape Verde - GIDTPiaget conducts applied research on control methodologies and interventions aimed at vectors of mosquitoes in the country (*Aedes aegypti, Anopheles arabiensis* and *Culex pipiens* sl).

Next, we present two pilot studies integrated into two priority lines of research; improvement of tools for monitoring the main and potential arbovirus vectors in the country and search for new control strategies aimed at the implementation of a surveillance system stratified by risk of arbovirus outbreaks.

Study area

Two pilot studies were carried out on Santiago Island, the largest of ten islands in the volcanic archipelago of Cape Verde and the most populated, with more than half the nation's population, 290,000 inhabitants out of 500,000 residents. Located here is the nation's capital, Praia, with 131,000 inhabitants and the focus for all vector-borne diseases (Fig 1.)

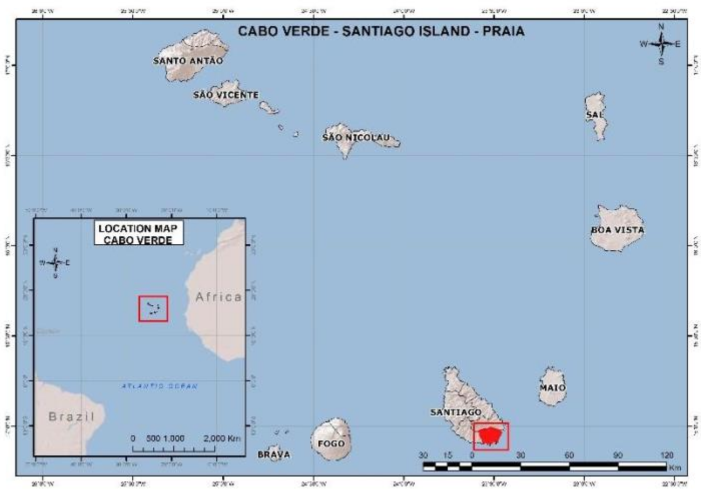


Fig 1. Cabo Verde, Santiago Island, City of Praia.

The country, located in the subtropical region 560 km west of the Senegal coast, has an arid and semi-arid climate, with an average annual temperature of 25º C and little rainfall, average annual precipitation below 250 mm, concentrated in the rainy season (July to October).

The island of Santiago is located in the southern island group or "Sotavento", of which Maio, Santiago, Fogo and Brava are part. The rest are included in the Barlovento group, located to the north and comprising the islands of Santo Antão, San Vicente, Santa Lucía, Sao Nicolau, Sal, and Boa Vista. With rugged terrain, Santiago has a rural population located in the interior of the island and an urban population, concentrated in the capital, which is growing rapidly, giving rise to the appearance of overcrowded neighbourhoods without planning or urban infrastructure and with poor housing conditions.

PILOT STUDY I

Assessment of the use of substances with attractive power in ovitraps as a method of monitoring *Aedes aegypti,* the vector of Z ika and dengue in Cabo Verde

The main strategies for vector monitoring and control based on methods for egg collection, larval research and adult mosquito collection. However, methods aimed at collecting mosquito eggs are more appropriate to the peculiarities of *Ae. aegypti* than larval research (Braga et al., 2000; Gómez et al., 2013). Oviposition traps (ovitrap) are the most sensitive and economical instrument for detecting Aedes species, especially when infestation levels are not revealed by larval indices (Acioli, 2006; Braga & Valle, 2007b).

To improve the efficiency of ovitraps, attractant substances such as organic matter infusions are used (Santos et al., 2003; Ministry of Health of Brazil, 2009a). Different studies report the *Bacillus turigiensis* var. *israeliensis* (Bti)-treated ovitrap technique in vector control as a promising strategy for detecting Aedes species (Santos et al., 2003; Tans *et al*., 2012; Stoops, 2005; Regis et al., 2008; Araújo et al., 2013; Lam et al., 2010). In addition to being a biolarvicide, there is evidence that Bti acts as a stimulant of Aedes species oviposition, improving the efficiency of ovitraps (Aronson & Shai, 2001; Santos et al., 2003; Stoops, 2005). In addition, household substances, like table salt, have been tested in ovitraps to control *Ae. aegypti* (Tilak et al., 2005).

The aim of this study was to evaluate the attractiveness of substances tested in ovitraps (salt, Bti and Bti + acacia infusion), as a complementary tool for monitoring *Ae. aegypti*, the zika and dengue vector, in the city of Praia.

Material and Methods

Attractive and repellent substances for Ae. aegypti

- VectobacG (granulated formulation of Bacillus thuringiensis var. israelensis serotype H14, 0.2 million IU/g, 0.2%
- Dilution to 1.2% of table salt

- 10% acacia infusion (100ml infusion for 1 litre of water). To prepare the infusion, 15 grams of acacia leaves were used *(Prosopis sp)* for each litre of water. The prepared solution rested for 7 days, before use, to allow for the fermentation of the organic material.

Simulated field study to evaluate the effectiveness of substances tested in ovitraps (Bti, Bti+infusion, Table salt) used to monitor Aedes aegypti

For this test, oviposition traps (OVTs) were used to monitor *Ae. aegypti* in Praia (ref), treated with Bti, Bti + infusion, and table salt. They were setup semi-randomly in eight georeferenced locations in the Palmarejo district, distributed in a homogeneous way (Fig.2).

Fig 2. Delimitation of the study area (Palmarejo) and the location of placed OVTs

Four OVTs were installed in each location containing: OVT Control (800ml tap water), OVT + Bti (0.62g), OVT + Acacia infusion (10%) + Bti and OVT + Salt (1,2%). These were placed outdoors, in the shade, with a distance of 2 to 3 meters between them (Reiter &Nathan, 2001; Acioli, 2006; Nunes 2013; WHO, 2011).

The OVTs were monitored weekly for three months (August-October 2014). Their contents were reviewed, and the four OVTs were rotated to pre-established points (A,B,C,D) within each location to eliminate bias. Every two weeks, the content of the traps were changed to ensure the effectiveness and attractiveness of the acacia infusion. Eggs were counted using a stereoscopic microscope (Lupa binocular Motic microscopes).

Weather data.

The National Institute of Meteorology and Geophysics (INMG) provided data relating to monthly abiotic factors (temperature, relative humidity and the rainfall).

Data analysis and calculation of rates of infestation and abundance of Ae. aegypti

Data obtained from the simulated field study was processed using Microsoft Excel. SPSS version 21 was used for descriptive and comparative statistical analysis of the data. A Kruskal-Wallis test was used to compare the attractiveness of different substances tested in the experiment, and to determine the degree of significance of the differences observed in the egg collections carried out in each point. A chi-square test was conducted to verify the existence of an association between the substances tested in the OVTs and the presence of larvae.

Two different indices to estimate the vector population density and its distribution were applied: The positive ovitrap index (POI), which is the proportion of ovitraps positive for the presence of Aedes eggs, and the density eggs index (DEI), the average number of eggs per positive ovitrap.

Results

During the three months of monitoring the OVTs, 45643 eggs of *Ae. aegypti* were collected. The POI was 90% and the DEI was 134. Analysing the distribution and abundance of the vector, October was the month with the highest indicators (Table 1).

Table 1: Total number of eggs of *Ae. aegypti*, number of positive OVTs, POI and DEI in the study period.

Meses	Nº de OVT analisada	Nº de OVT positiva	Nº de ovos	IPO (%)	DEI
August	123	109	14079	88,62	129,17
September	127	111	13937	87,40	125,56
October	127	120	17621	94,53	145,69
Total	377	340	45643	90	134

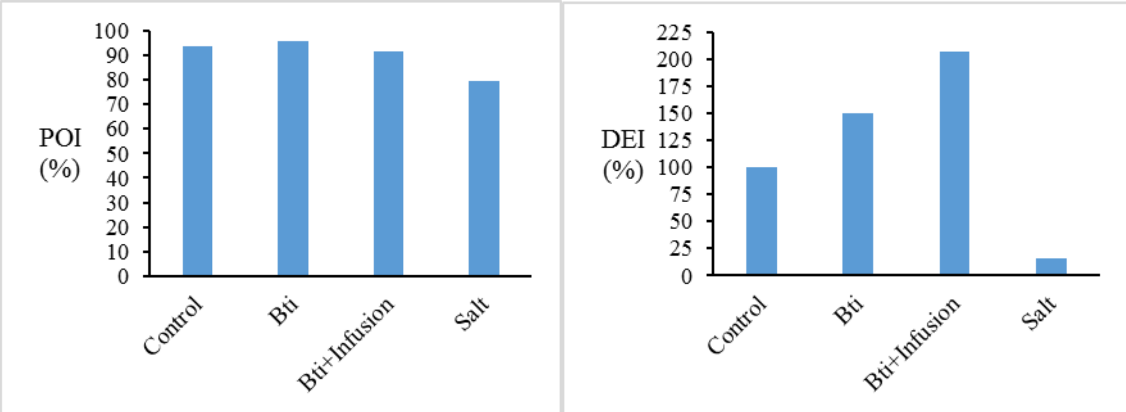
From the individual analysis of each of the eight locations, the presence of *Ae. aegypti* eggs was observed in all collections carried out, with differences in the amount of eggs collected in different locations, not following a distribution pattern over the period of this experiment (Table 2).

Table 2: Total number of *Ae. aegypti* eggs, number of positive OVTs, POI and DEI, by localities.

Locations	Nº OVT analyzed	Nº OVT positive	Nº eggs	POI (%)	DEI
1	47	46	6512	97,87	141,57
2	46	42	7912	91,30	188,38
3	48	46	6288	95,83	136,70
4	45	33	2418	73,33	73,27
5	48	46	5766	95,83	125,35
6	47	41	4087	87,23	99,68
7	48	41	4691	85,42	114,41
8	48	45	7969	93,75	177,09
Total	377	340	45643	90	134

A comparative analysis (Kruskal-Wallis test) of the turnover of OVTs, at the four points in each location, shows that only location 6 (P = 0,012) showed a statistically significant difference ($P < 0.05$) in the number of eggs found at each point. After applying the Tukey test at location 6, it observed a significant difference for eggs collected between points A and B ($P < 0.05$). As a whole, the result of the comparative analysis demonstrated a distribution of OVTs, by zones, without influence of the location of OVTs in the oviposition of females of *Ae. aegypti*.

Analysing the POI obtained according to the type of substance tested in the OVTs, the differences were not appreciable except for the OVTs with salt in relation to the control (Figure 3A). For the DEI the differences were notable. The OVTs with Bti + infusion collected a greater amount of *Ae. aegypti* eggs (20233) with a DEI of 229.92. The OVTs with table salt collected the lowest number of eggs (1508), having recorded a DEI of 16.39 (Fig 3.B)



A B

Fig 3. Ovitrap Positivity Index (IPO) and Density Eggs Index (DEI) of the substances tested in OVTs used to monitor *Ae. aegypti* in the Palmarejo district.

The DEI of the tested substances is presented in relation to the number of eggs in the control, taking the value of 100%.

Comparison of the different OVTs tested with the Control OVT (Kruskal-Wallis test) in relation to the number of eggs collected during the study period demonstrates that there is a statistically significant difference ($P < 0.05$) between them and the Control OVT ($P = <0,001$).

Regarding temperature and monthly relative humidity, there were no major variations during the study period, with mean values of 26.9°C and 82% respectively. Average monthly rainfall was 37.7 mm, occurring almost entirely in September (108.8 mm). There is no significant relationship between climate data and infestation rates (POI and DEI).

Discussion

In this study, it was observed that the adaptation of the ovitrap from the model developed by Fay & Eliason (1966) is promising for monitoring *Ae. aegypti* in the city of Praia, since the use of acacia infusion + Bti in the OVT collected twice as many eggs when compared to the use of tap water alone. The use of acacia infusion in OVTs is an innovation, used for the first time (in oviposition traps) in this study. The use of table salt in OVT worked as a repellent for female *Ae. aegypti* mosquitoes in Cape Verde.

Conclusion

Of the substances tested in ovitraps, Bti and Bti+infusion work as attractants of *Ae. aegypti* from the city of Praia, whilst table salt having been verified as having an effect of repellency. The use of biological larvicide Bti, in ovitraps, allowed the permanence of OVTs in the field, safely, for at least 15 days. Monitoring with ovitraps in the

Palmarejo neighborhood showed that it had a high infestation of *Ae. aegypti*, during the study period.

PILOT STUDY II

BR-OVT evaluation as a tool for monitoring mosquito vectors in Cape Verde

Cape Verde has a record of eleven species of Culicidae in the country, two of which are recognized vectors, *Ae. aegypti* and *Anopheles arabiensis* (Alves et al., 2010, Alves et al., 2014). This fact does not mean that in the future other potential vector species, such as *Culex pipiens* and *Culex quinquefasciatus*, will not be able to transmit other diseases in the country, such as West Nile.

Currently, the vector control program does not monitor the vectors in space and time, limiting itself to intervention measures, with intra-household spraying (PID) (deltamethrin) for adults, and with temephos and diesel for the treatment of breeding sites. Larvivorous fish are also used as biological control agents in irrigation water tanks (Ministério da Saúde de Cabo Verde, 2012).

Experiments have been conducted in the country of monitoring populations of *Ae. aegypti* with ovitraps (Ref), but it is necessary to develop and adapt tools that serve, at the same time, to monitor and control potential vectors in the country.

Methods that use biolarvicides (Bacillus thuringiensis israelensis - Bti) with the BR-OVT trap, for Culex, have proven to be efficient, safe and operationally simple (SANTOS et al., 2003; REGIS et al., 2008; BARBOSA; REGIS, 2011). This composition (BR-OVT and Bti) forms a continuous monitoring system, allowing the trap to remain in the field for months, and works as a good strategy to attract and eliminate mosquitoes (BARBOSA, 2007). Because of a project carried out in the Metropolitan Region of Recife (RMR), Pernambuco, Brazil, the BR-OVT proved to be able to remove significant amounts of *Cx. quinquefasciatus* rafts from the environment (BARBOSA, 2007; BARBOSA; REGIS, 2011).

The aim of this study was to evaluate the efficiency of adhesive BR-OVT as a tool for monitoring the populations of *Culex pipiens* s.l. and *Ae. aegypti* in the main urban centres of the island of Santiago - Cabo Verde.

Material and Methods

Study area

The pilot study was carried out between July and August 2014 in two cities on the island of Santiago, Cape Verde: Praia (14° 55' N 23° 30' W) and Assomada (15° 05′ 45″ N 23° 40 ′ 00″ W). The country's capital is located at sea level and has large urban agglomerations. Considered like a capital of the island's interior, the city of Assomada is predominantly rural, is located at 44 km from the capital and situated on a plateau at 391 m above sea level.

Study tool

*M*osquitoes were collected using the BR-OVT trap, developed by Barbosa et al. (2007) and adapted to collect adult mosquitoes by Xavier et al. This tool, made up of a black polyethylene box (13 x 35 x 24 cm), with a central opening (16 x 9 cm) on the upper face and containing, inside, a black container with a capacity of 4 L. Around this, there is an adhesive edge on the top face and a substrate (fabric) attached to the edge inside the container. BR-OVT is installed indoors (Fig 4.)

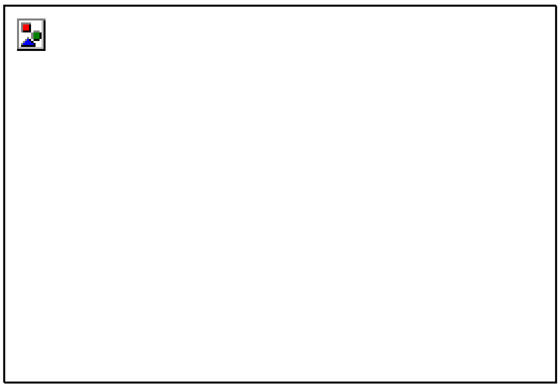


Fig 4. BR-OVT adhesive trap.

Experimental approach

For the development of this project, 40 indoor traps were installed, of which 25 in Praia and 15 in Assomada. In each trap, an attractive solution of 500 ml of acacia infusion with 3 L of water was added. Each BR-OVT adhesive was treated with 2 g of the biolarvicide VectoBac G, based on *Bacillus thurigiensis* var. *israelensis*, from Valent BioScience. Every 30 days the adhesive edges and the oviposition substrate for *Ae. aegypti were replaced*. The rafts of *Culex pipiens were* retrieved once a week and counted. All mosquitoes captured on adhesive edges and ovipositor substrates were taken to the laboratory of the Tropical Disease Research Group at the Jean Piaget University in Cape Verde, for identification and quantification.

Results

During the study period in Praia and Assomada, 382 adult mosquitoes were collected, belonging to the species *Cx. pipiens s.l.* and *Ae. aegypti.* Of these, about 90% were captured in Praia.

During the same study period, 207 rafts (Culex eggs) were also collected, the majority (88%) in the city of Praia.

In Praia, from the total number of mosquitoes captured, 72% classified as *Cx. pipiens* s.l. and the rest as *Ae. aegypti*. Mosquito densities obtained in the two months showed little variation when compared. However, for egg collection, these traps presented results opposite to those described in Brazil: the positivity for Aedes eggs was higher than for Culex eggs (rafts), with positivity of 96% and 40% for the respective species.

In Assomada, the collection of adult mosquitoes in adhesive BR-OVT was lower than in Praia and about 50% of these traps were positive for adult mosquitoes, in both months. As in Praia, mosquito densities showed little monthly variation. However, of the total number of mosquitoes collected, there was a greater presence of *Ae. aegypti*

(60%) captured over Cx. pipiens s.l. (40%). As for the presence of eggs, the positivity of adhesive BR-OVT was 75% for Aedes, and 25% for Culex (Fig 5.).

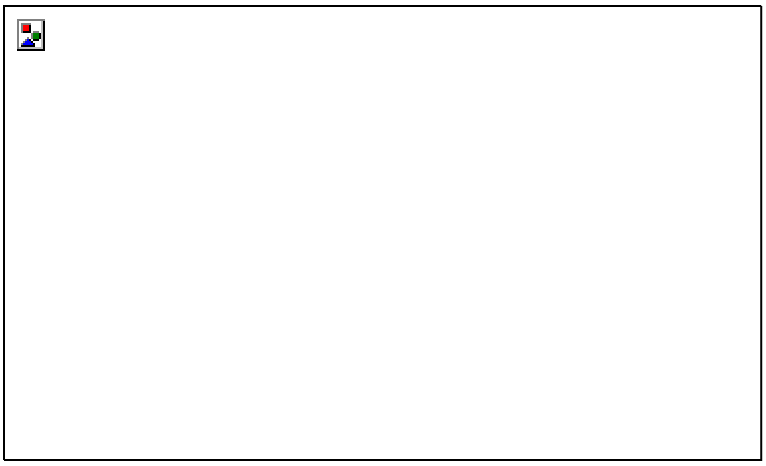


Fig 5. Percentage of positivity of the BR-OVT trap for Aedes and Culex eggs, per month, in the cities of Praia and Assomada during July and August 2014.

Discussion

The results showed that BR-OVT adhesive can be a good tool for monitoring the two species in Praia, Cape Verde. Although it was developed to capture *Cx. quinquefasciatus*, this trap also captures *Ae. aegypti* at different stages of its cycle, both in Praia and Assomada.

The presence of *Cx. pipiens* s.l. is associated with infusion activity, with a drop in the number of rafts collected over the months of observation. In contrast, *Ae. aegypti* colonizes BR-OVT as the infusion loses its activity, given the known preference of this species for breeding sites with a low concentration of organic matter. Another factor that contributes to the collection of *Ae. aegypti* is its population size. According to Alves et al. (2010), who carried out an entomological survey of the culicidological fauna of the leeward islands of Cape Verde, this is the prevalent species at the expense of others.

Conclusion

Adhesive BR-OVT is a potentially employable trap for monitoring *Cx. pipiens s.l.* and Ae. aegypti on the island of Santiago, Cabo Verde, as it is sensitive to the presence of different stages of the life cycle of both mosquitoes.

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