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Reproduction of mosquitoes (Diptera: Culicidae) in Santa Cruz, Santiago island, Cape Verde Islands

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ABSTRACT

Mosquitoes are dipterous insects with an important role in the transmission of diseases like malaria and dengue. During a dengue fever outbreak in the Cape Verde Islands in 2009, several studies were undertaken to support vector control. The present study was carried out in the district of Santa Cruz, Santiago island, to evaluate previous measures taken to control mosquito populations. Results show that mosquitoes use domestic water containers to breed. Barrels, drums and pots were all used. Of these, drums were most frequently found being infested with mosquito larvae or pupae. Morphological identification showed that *Anopheles arabiensis* and *Aedes aegypti* were present in the study area. *Ae. aegypti* was the commonest of the two and the only one found throughout the study area. Results show that socio-economic factors influence container positivity.

RESUMO

Os mosquitos são insectos dípteros que têm um papel importante na transmissão de doenças como o paludismo e a dengue. Durante uma epidemia da dengue em Cabo Verde em 2009, foram desenvolvidos estudos no sentido de dar suporte ao controlo vectorial. Este estudo foi desenvolvido no Concelho de Santa Cruz, ilha de Santiago, para avaliar o efeito das medidas de controlo tomadas sobre populações de mosquitos presente na área. Os resultados mostraram que os mosquitos utilizam recipientes domésticos para a reprodução. Dentre esses, os mais frequentes foram os barris, os bidões e os potes, mas os bidões foram os que mais se apresentavam com presença de larvas ou pupas dos mosquitos. A identificação morfológica mostrou que *Anopheles arabiensis* e *Aedes aegypti* está presente na área de estudo, embora somente *Ae. aegypti* foi encontrado em todas as localidades de estudo. Supomos que factores socio-económicos têm um papel importante na positividade dos recipientes.

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INTRODUCTION

Mosquitoes are dipterous insects with potentially important medical implications. They belong to the Culicidae, which include three subgroups, viz. Anophelinae, Culicinae and Toxorhynchitinae (Consoli & Oliveira 1994). In the Cape Verde Islands, the first mosquito species, *Anopheles gambiae s.l.*, was reported from Santiago island in 1909 (Ribeiro *et al*. 1980). Today, 10 species, belonging to two subgroups (Anophelinae and Culicinae) and four genera (*Anopheles*, *Aedes*, *Culex* and *Culiseta*), are known to be present in the archipelago, of which *Anopheles arabiensis* Patton, 1905, *Aedes aegyti* Linnaeus *in* Hasselquist, 1762 and the *Culex pipiens* complex are the most important in relation with public health concerns (Alves *et al*. 2010).

In the Cape Verde Islands, malaria and dengue are the main diseases transmitted by mosquitoes. Malaria is one the principal vector-borne diseases, being transmitted by female mosquitoes of the genus *Anopheles*. The World Health Organization (WHO) has estimated that worldwide 3.3 billion people were at risk of acquiring malaria in 2010, while of all geographical regions, human populations in sub-Saharan Africa carry the highest risk of acquiring malaria (WHO 2011). Dengue fever is a mosquito-borne infection (arbovirose) that in recent decades has become a major international public health concern and its frequency has grown dramatically around the world. Two species (*Ae. aegypti* and *Ae. albopictus*) have been identified as the major vectors of dengue and it is currently estimated that there may be 50 million dengue infections worldwide every year (WHO 2012).

The first dengue epidemic in Cape Verde occurred in 2009 and started at the same time as an outbreak of pandemic influenza A (H1N1). After virus confirmation, the Cape Verde government established a ministerial committee on dengue control and clinical management. Vector control and social mobilization were the main measures taken to bring the dengue outbreak under control (WHO 2009). Vector control was based on treatment of mosquito breeding sites with Temephos (organophosphate) and adult longevity reduction by using indoor residual spraying (IRS). In October 2009, a team from WHO's Regional Office for Africa arrived to support the Cape Verde authorities in investigating the outbreak. Several studies were carried out in the archipelago to understand the transmission cycle of dengue and to support vector control. The present study was carried out in the Santa Cruz district of Santiago island in order to evaluate measures previously implemented in vector control (larvae control and adult longevity reduction) in mosquito populations.

METHODS

The study was carried out at five localities of the district of Santa Cruz: Achada Bel-Bel, Cancelo, Santa Cruz, Achada Fazenda and Renque Purga (Fig. 1). Santa Cruz district is a municipality located in the eastern part of Santiago island with around 32,965 inhabitants and an area of *ca.* 150 km², i.e. *ca.* 205 hab/km² (Instituto Nacional de Estatística 2010). The seat of the municipality is in the town of Pedra Badejo. The district is generally arid and no natural standing surface fresh-water is present.

During May 2010, a variety of water containers (both indoors and outdoors) at human and animal dwellings was inspected and catagorized as barrel, drum, fountain, pot, tank, vase or ‘other’ (Table 1). When present, mosquito larvae and pupae were collected using standard methods, stored in tubes and labeled with date, locality, container type and placement (indoors or outdoors human or animal dwelling). Adults were collected using manual aspirators and stored in individual tubes and labeled as for larvae and pupae. Before identification, larvae were killed using alcohol 70º. Adults collected or emerged from pupae were killed at low temperatures (~ -3ºC). Morphological identification was conducted at the *Núcleo de Pesquisas em Ciências Aplicadas* of the University of Cape Verde in Praia according to Ribeiro *et al.* (1980).

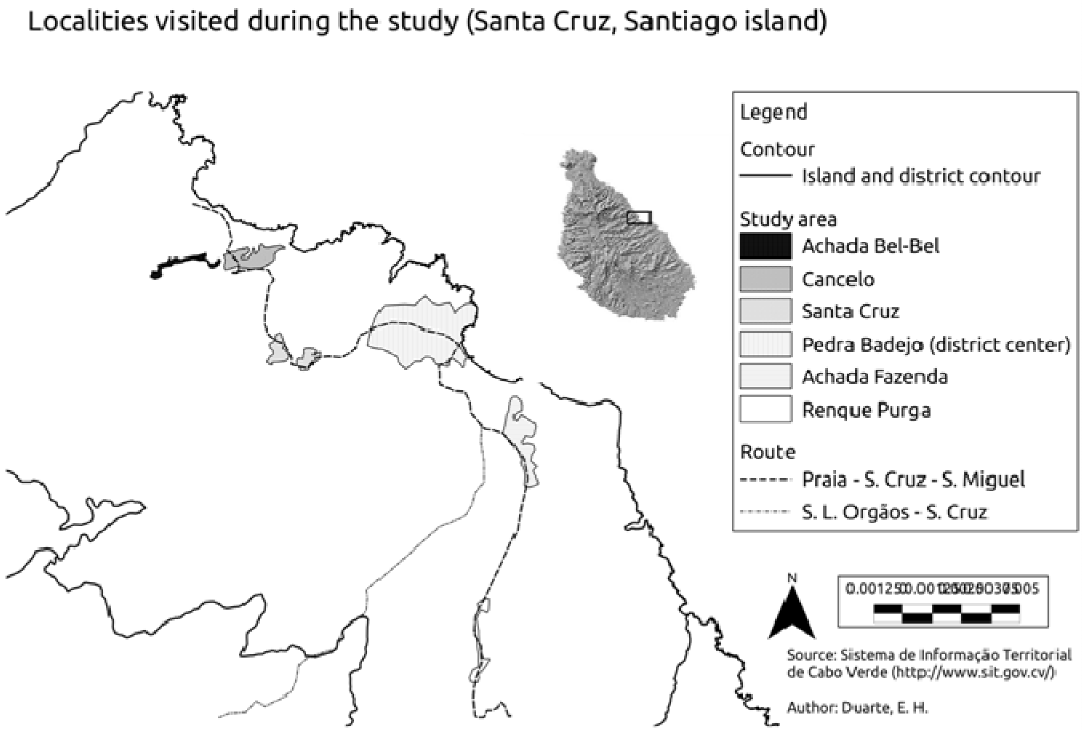

Fig. 1. Santa Cruz district, Santiago island, Cape Verde Islands. The study area is located to the north and south of the town of Pedra Badejo.

	Water container						
	Barrel	Drum	Fountain	Pot	Tank	Vase	Other
Volume (in litres)	~200	~200	~1	5	2,000	<1	2-3
Characteristics	Plastic	Iron	For domestic animals			For flowers	

Table 1. Types of water containers found during this study and some of their characteristics.

Observed frequencies were compared using goodness of fit tests. To model the effect of different parameters in container positivity, a logistic regression was used. Larvae and pupae density was summarized as present (at least one larva or pupa per container) or absent (no larvae or pupae recorded). The probability to record at least one larva or pupa per container was measured using as response variable the number of human residents per habitation, the number and type of water containers, their placement (indoors or out-doors human or animal dwellings), the locality within the study area and their interactions. The final model was written as $\log\ (p/1-p)=b_0+b_1x_1+b_2x_2+...+b_nx_n$, were b_0 represent the intercept of the model, b_1,b_2 and b_n the regression coefficients and x_1, x_2 and x_n the response variables. Significant differences were established at 0.95 confidence level (a=0.05). Statistical analysis was carried out using R version 2.13.1 (R Development Core Team 2010).

RESULTS

During this study, 110 dwellings were visited and 345 water containers inspected (Table 2). Barrel (39.13%), drum (27.83%) and pot (17.68%) were the containers most frequently found and a significant difference was observed between their observed frequencies (χ^2=312; df=6; p<0.001). Half (50.72%) of the water containers were located at Renque Purga and Achada Fazenda in southern Santa Cruz and a significant difference was found in frequency of water containers in different parts of the study area (χ^2=22; df=4; p<0.001). In 7.54% (n=26) of the water containers inspected, mosquito larvae or pupae were present, with drums being positive most frequently. Breeding sites of mosquitoes were located mostly indoors (57.69%) at human and animal dwellings, although without significant difference (W=8153; p>0.05). *Ae. aegypti* was the taxon most frequently encountered and the only one found throughout the study area (Fig. 2). *An. gambiae s.l.* was only found in Santa Cruz and Cancelo. Other Culicinae (not *Ae. aegypti*) were registered at three localities (Fig. 2), but these were not identified as to species.

Locality	Number of inspected dwellings	Number of water containers	% Positive container (n)
Achada Bel-Bel	20	68	2.94 (2)
Achada Fazenda	30	81	8.64 (7)
Cancelo	20	59	3.39 (2)
Renque Purga	20	94	13.83 (13)
Santa Cruz	20	43	4.65 (2)
Total	110	345	7.54 (26)

Table 2. Water containers with mosquito larvae and pupae in the study area.

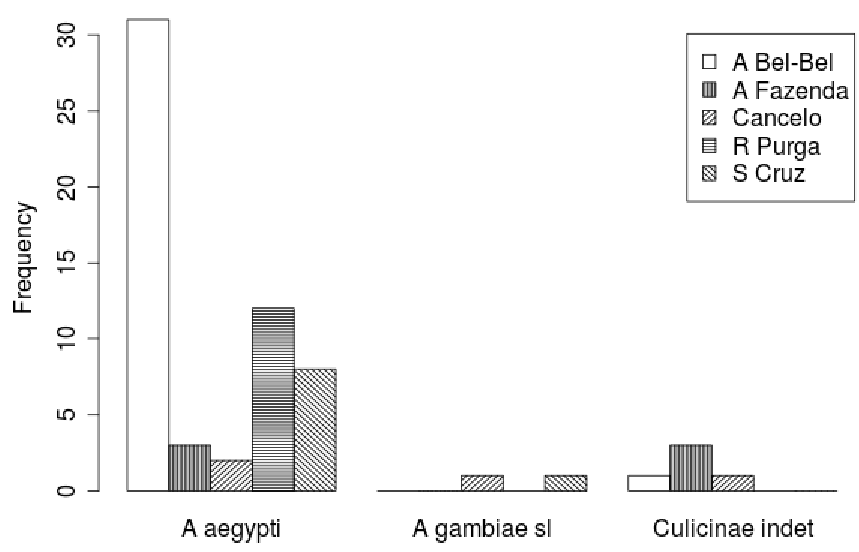

Fig. 2. Frequency of mosquito species collected at different localities based on identification of larvae, pupae and adults.

Table 3. Logistic regression for container positivity using different parameters and their interaction. The best model was selected using Akaike Information Criterion (* no data recorded, ** not estimated).

	Estimate	OR	CI	p-value
Intercept	-1.71	**	**	>0.05
Container number	-0.47	0.13	0.03 – 0.51	<0.05
Container localization	-0.47	0.28	0.06 – 6.78	>0.05
Container type				
Fountain	-18.2	<0.001	**	>0.05
Drum	1.21	3.36	0.77 – 14.71	>0.05
Others	-18.5	<0.001	**	>0.05
Pot	-79.4	0.45	0.04 – 4.73	>0.05
Tank	1.28	3.6	0.22 – 59.53	>0.05
Vase	2.57	13.03	0.69 – 244.7	>0.05
Locality				
A. Fazenda	-0.81	2.25	0.31 – 16.37	>0.05
Cancelo	0.01	1.01	0.11 – 9.01	>0.05
R. Purga	2.85	17.21	2.62 – 112.85	<0.05
S. Cruz	-0.34	0.71	0.08 – 6.45	>0.05
Interaction				
Fountain	*	**	**	**
Drum	2.16	8.67	0.52 – 142.75	>0.05
Others	0.94	2.56	**	**
Pot	-0.16	<0.001	<0.001	>0.05
Tank	-0.18	<0.001	<0.001	>0.05
Vase	-0.19	<0.001	<0.001	>0.05

Table 3 summarizes the logistic regression modeling the effect of different parameters in container positivity. Container number and type, locality within the study area and relation between container type and locality were the parameters which affected container positivity.

DISCUSSION

The commensal existence of some mosquito species within the human environment is well-known. Commensal taxa usualy breed in water containers used by man in his daily activities. Many types of containers have been reported as potential breeding sites for mosquitoes (cf. Consoli & Oliveira 1994, Natal 2002). During the present study, no abandoned water containers were found. The absence of these could be the result of the clean-up operations carried out in 2009 (one of the measures included in social mobilization) or simply due to the lack of rain during the study period. This result suggests that a continuous transmission cycle can be maintained by mosquito populations by breeding in domestic containers. For an effective control of these mosquitoes, at least two spraying programmes per year need to be conducted. Although barrels were the most frequent water container, drums were the most used as breeding site. Drums were often found to be the container type in an advanced state of degradation (Fig. 3) and conservation status of the container may be correlated with their positivity as a breeding site for mosquitoes, a hypothesis to be confirmed or rejected in future studies.

Indoor breeding sites were more frequent than outdoor sites, although no significant difference was observed at the 0.95 confidence level (W=8153, p>0.05). However, elsewhere in Cape Verde, outdoor breeding sites were more frequent than indoor sites (E.H. Duarte *et al.* unpublished data). We presume that these differences in placement are related to the locally prevailing social and cultural customs.

During this study, 63 specimens (57 larvae and six adults) were morphologically identified. Due to the limited number of adult specimens, larvae and adults were pooled in the results. *Ae. aegypti* was the only species found throughout the study area (Fig. 2). Adaptation of this species to the human environment is well documented (cf. Ribeiro *et al.* 1980, Consoli & Oliveira 1994, Natal 2002). By breeding in artificial containers, this species consolidates a good transmission cycle (Natal 2002). *An. gambiae s.l.* was only found in Santa Cruz and Cancelo (Fig. 2). *An. arabiensis* is the only member of the *An. gambiae* complex known to be present in Cape Verde (Cambournac *et al.* 1982). This species is the main malaria vector in sub-saharan Africa and in Cape Verde it has been identified as vector of lymphatic filariasis in Santiago island (Franco & Menezes 1955).

Three possibilities may explain the distribution of taxa found in this study: i) insufficient sampling effort (i.e. number of inspected dwellings; Table 2); ii) breeding sites of *An. arabiensis* were not inspected (cf. Ribeiro *et al.* 1980); iii) the species is really absent at other localities. Other species were found at some localities, but not identified at the species level and these are given as Culicinae indet. (Fig. 2). As these bred in domestic containers, we suppose that they represent (at least in part) members of the *Cx. pipiens* complex, a species-group well-adapted to the human environment (Ribeiro *et al.* 1980).

Only two parameters evaluated in the logistic regression did not present any effect in container positivity. Container placement, when tested alone, showed no effect in container positivity (ANOVA, $p>0.05$), but in interaction with container type, a moderate significant effect was demonstrated ($p<0.05$). This interaction augments the drums odds-ratio (OR) of 3.36 to 8.67, when compared with barrels (OR=1). We believe that this interaction is caused by socio-economic factors, because this interaction represents the number of containers indoors or outdoors of

Fig. 3. Degraded drums, Achada Bel-Bel, Santa Cruz, Santiago, 11 December 2010 (António Varela).

dwellings. It was noted that the presence of outdoor containers was correlated (not statistical tested) with the presence of domestic animals, remodelling of the dwellings and other social concerns. As mentioned by Arunachalam *et al.* (2010), vector breeding and reproduction of *Ae. aegypti* are influenced by a complex interplay of factors. Some of these factors were taken into account during this study, but the influence of socio-economic factors was not tested. Although our results provide evidence for an important role of socio-economic factors in container positivity, more conclusive studies are needed to support this hypothesis.

In conclusion, the lack of data from before the 2009 dengue epidemic fever outbreak makes it difficult to evaluate the methods employed herein in relation to reproduction in mosquito populations. However, our results indicate the continuing breeding of mosquitoes in domestic containers after control measures had been implemented. Future studies should investigate why and how mosquitoes are able to use these containers as breeding sites even after control measures have been taken.

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