ORIGINAL ARTICLE

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STUDY OF OVIPOSITION BEHAVIOR OF *Aedes aegypti* IN TWO NEIGHBORHOODS UNDER THE INFLUENCE OF SEMI-ARID CLIMATE IN THE MUNICIPALITY OF SALINAS, STATE OF MINAS GERAIS, BRAZIL

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ABSTRACT

The mosquito *Aedes aegypti* (Diptera: Culicidae) is the main vector of human arborvirus in Brazil. Oviposition behavior is important for the survival and dispersal of *A. aegypti*. The objective of this study was to evaluate the oviposition behavior and its relationship to meteorological factors. Twenty ovitraps were installed in two neighborhoods in the urban area of the city of Salinas, MG, southeastern Brazil. The traps were inspected weekly for the presence of eggs in the water and on the paddle. Simultaneously, regional weather data were analyzed. The results showed the Ovitrap Positivity Index (OPI) varied greatly, from 0 to 90%, between the weeks and boroughs sampled, demonstrating the vector's great population fluctuation. Among the analyzed climatic factors, rainfall strongly influenced oviposition, followed by relative air humidity. The number of eggs found on the paddle was significantly higher than in the water ($p<0.05$). However, a significant increase of eggs in the water was noticed when the relative humidity dropped below 40%. This seems to be an important behavior to maintain the population in periods of drought. Through the low number of eggs (<30) found in 56% of positive traps, we concluded that skip-oviposition behavior occurred, contributing to the prevalence of mosquitoes during the sampled period. It is concluded that the occurrence of water oviposition may be higher than described in the literature, and should be studied due to its evolutionary and epidemiological importance.

KEY WORDS: *Aedes aegypti*; climate; behavior; oviposition.

RESUMO

Estudo do comportamento de oviposição do *Aedes aegypti* em dois bairros sob a influência do clima semiárido no município de Salinas, Minas Gerais, Brasil

O mosquito *Aedes aegypti* é o principal vetor de arborvírus humanos no Brasil. O comportamento de oviposição é importante para a sobrevivência e dispersão do *A. aegypti*. O objetivo deste

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trabalho foi avaliar estes comportamentos e sua relação com fatores climáticos. Instalaram-se vinte armadilhas ovitrampa em dois bairros de Salinas-MG. Todas foram inspecionadas semanalmente para contagem e identificação dos ovos presentes na água e na palheta. Paralelamente, analisaram-se dados meteorológicos da região. Os resultados mostraram que o Índice de Positividade de Ovitrampa (IPO) variou muito (0% a 90%) entre as semanas/bairros amostrados, o que demonstra a grande flutuabilidade populacional do vetor. Dentre os fatores climáticos analisados, o que mais intensamente influenciou a oviposição foi a precipitação, seguida da umidade relativa. O número de ovos encontrados na palheta foi significativamente maior que na água ($p<0,05$). Entretanto, observou-se aumento significativo de ovos na água quando a umidade relativa esteve abaixo de 40%. Este parece ser um dado importante para a manutenção das populações em períodos de seca. O baixo número de ovos (<30) encontrados em 56% das armadilhas positivas demonstrou a ocorrência do comportamento de *skip-oviposition* que pareceu contribuir para a prevalência do mosquito em todo o período amostrado. Concluiu-se que a ocorrência de oviposição na água pode ser maior do que o descrito na literatura e deve ser investigada em virtude de sua importância evolutiva e epidemiológica.

DESCRITORES: *Aede aegypti;* clima; comportamento animal; oviposição.

INTRODUCTION

Aedes aegypti Linnaeus, 1762 (Diptera: Culicidae) is a cosmopolitan and highly anthropophilic mosquito (8, 29). This insect is the main vector of dengue virus, which is considered the most important arborviral disease of our time and a major global public health problem (21, 29). Recently, this vector has gained new importance, since it may be responsible, along with *A. albopictus*, for the autochthonous transmission of Chikungunya fever in Brazil. Up to week 42 of the study (12/10 to 18/10/2014), 682 autochthonous cases were confirmed and more than 900 are under investigation (22).

In Brazil, *A. aegypti* is always associated with human households and the peridomicile, where it finds food and oviposition sites in abundance (21). Oviposition behavior has extreme importance in the lives of these insects and is closely related to their survival and the epidemiology of the diseases they transmit (31). Pregnant *A. aegypti* females are highly selective in choosing oviposition sites (24, 31, 32).

Besides the high selectivity in the choice of breeding sites, these insects distribute their eggs over several sites, behavior named as "skip-oviposition" (2, 15, 24). This can reduce the risk of loss of all offspring because of site elimination (24). This behavior is also directly related to the geographic distribution of the vector, which ultimately spreads the dengue virus in the human population (11, 15).

Despite oviposition directly into the water being common in many Culicidae – e.g: Subfamilies Culicinae and Anophelinae (4) – many authors assert that the *Aedes aegypti* mosquito rarely lays its eggs on the water surface, and report that only 3% of the eggs are laid directly on the water (5, 7, 8, 13). Because of this, surveys of entomological indices obtained from oviposition traps (Ovitrap) discard the water of the traps without any analysis (21). However, in a study conducted in Brazil under laboratory conditions, the percentage of eggs in the water was higher than 40% (20).

Therefore, many aspects of the oviposition behavior of these insects are still controversial. Furthermore, little is known about the influence of climate on this behavior. The present study arises from the need to evaluate the actual oviposition behavior of *A. aegypti*, especially in the breeding sites used for monitoring dengue in Brazil. Given the above, the objective was to evaluate the oviposition behavior of *A. aegypti* in ovitraps and its relationship with climatic factors in a semi-arid region to the north of Minas Gerais.

METHODS

The study was conducted in Salinas, located 42°29'W, 16°17'S, in the north area of Minas Gerais, Brazil. The city had a population of 30,716 inhabitants in the urban area in 2010 (16), 729 blocks, and is located between the biomes of Cerrado and the Atlantic Forest. The region is part of the semiarid area of Minas Gerais and has an average annual rainfall of 904 mm (http://cidadesnet.com/municipios/salinas.htm), which passes through a remarkable dry period - April to October - with poorly distributed rains, and another period of torrential and sporadic rains - from November to March (17, 18). These climatic conditions, together with the presence of the laboratory infrastructure of the Instituto Federal do Norte de Minas Gerais (IFNMG - Salinas) determined the choice of the municipality for this research.

Twenty ovitraps were made by hand from recyclable materials - plastic bottles - cut to a height of 13 cm and externally painted black. The trap followed the parameters initially developed by Fay & Perry (1965) and improved by Fay & Eliason (1966), and consisted of a container of black and matte color containing 300 mL of tap water. Inside, a substrate for oviposition was mounted vertically (plywood paddle, dimensions 10x3 cm), with the rough surface exposed, as shown in Figure 1.

These traps were placed in two neighborhoods of the city - São Geraldo (A) and São José (B), in the peridomicile of the chosen residences, sheltered from the rain. Residents were informed about the project and consented to its installation for the entire sample period. There are approximately 30 blocks in each neighborhood, which results in three blocks per trap, on average. The distribution (Figure 2) occurred in a way that was as homogeneous as possible in an attempt to cover sampling the entire area of the neighborhood, and was influenced by the availability of residents and the presence of a proper peridomicile for installation of the ovitraps (10). Neighborhood A (traps numbered 1 to 10), is central and more urbanized, located near the River Salinas. Neighborhood B (traps numbered 11 to 20) is peripheral and contains unpaved streets and vacant lands.

Data collection occurred for 25 weeks between the months of June 2012 and January 2013. Due to operational problems, seven weeks were not sampled in this interval. The traps were inspected once a week. The water and paddle contained in each of the 20 ovitraps were collected, individually labeled and transported to the Laboratory of Biology of the Instituto Federal do Norte de Minas Gerais - *Campus*

Salinas. This routine was different from that recommended by the Ministry of Health in Brazil (21), since the presence of eggs in water from ovitraps was also analyzed.

Figure 1. Picture of the Eco-ovitrap manufactured by hand from a plastic bottle for the study.

In the laboratory, the eggs in the water and on the paddle of each ovitrap were counted with the aid of a stereoscopic microscope. The paddles and positive water samples were separately placed in trays for hatching. After hatching, the larvae L3 were identified to confirm the species *A. aegypti*.

Figure 2. Georeferenced map indicating the location of the traps in the two sampled neighborhoods.

From the recorded number of eggs, the following entomological indices recommended by the Ministry of Health were calculated: "*Ovitrap Positivity Index* (OPI) = number of positive traps x 100/number of inspected traps", which shows the mosquitoes' spatial distribution, and "*Egg Density Index* (EDI) = number of eggs /

number of positive traps" which indicates the vector's population abundance (14, 21). However, unlike the recommended routine, these indices were calculated also considering the number of eggs found in the water of the trap.

Meanwhile, the meteorological data of the municipality of Salinas provided by the Weather Station of Salinas through the National Institute of Meteorology (INMET) website were analyzed in an attempt to correlate the climatic variables - temperature, relative humidity (RH) and rainfall - with the values of oviposition found on the paddle and in the water of the ovitraps.

Statistical analyses were made as follows: To compare the number of eggs deposited in each neighborhood, data were submitted to the Anderson-Darling Normality Test at a significance level of 95%. Means with normal distribution were compared by paired *t* Test. When distributions were not in accordance with the criteria of normality, medians were compared using the Mann-Whitney test.

To evaluate the amount of eggs deposited in the water and on the paddle, the percentage of eggs found in each location, in each sampled week, and in each of the neighborhoods was used. The data were transformed into arcsin and the comparison of means followed the same methodology described above. To check the influence of the climate on the deposition of eggs in the water, we compared the percentage of eggs deposited in water in the weeks that showed relative humidity below 40% with those where this parameter was over 40%, using the Mann-Whitney test. Furthermore, the associations between climatic variables and the entomological indices collected were analyzed by Pearson's correlation coefficient (ρ) (23). All tests were performed in the Minitab 16 program.

RESULTS

During the experiment, a total of 18,473 eggs were collected in the 25 weeks sampled. There was capture of eggs in every week.

The differences among the sampled neighborhoods (paving, urbanization and location) did not seem to influence the oviposition of *A. aegypti*, since there was no significant difference in the number of eggs deposited in the neighborhoods A (6,916) and B (11,557). Also, there was no significant difference in the amount of eggs deposited in the water and on the paddle, between neighborhoods.

The indices obtained through the oviposition traps (OPI and EDI) varied greatly between weeks and sampled neighborhoods (Figures 3 and 4). The OPI ranged from 0 to 90% whereas EDI ranged from 0 to 1196.

It was possible to observe that in the majority of weeks of the study, especially between weeks 24 and 36, there was an alternation among the scores obtained in the neighborhoods (Figure 3).

Significant associations ($p<0.05$) were obtained between the entomological indices and the following climatic factors: maximum, minimum and weekly average RH; and weekly total rainfall. The highest Spearman correlation coefficients were observed between the total number of weekly eggs *vs* Rainfall (ρ = 0.680); Between EDI *vs* Rainfall (ρ = 0.820) (Figure 4); and between the OPI *vs* maximum weekly RH (ρ = 0.548). The OPI found in neighborhood A was the only parameter that was not climate-related, indicating that other causes of variation have contributed in a more prominent way than the climatic factors analyzed. The temperature did not interfere significantly in the rates of oviposition in Salinas.

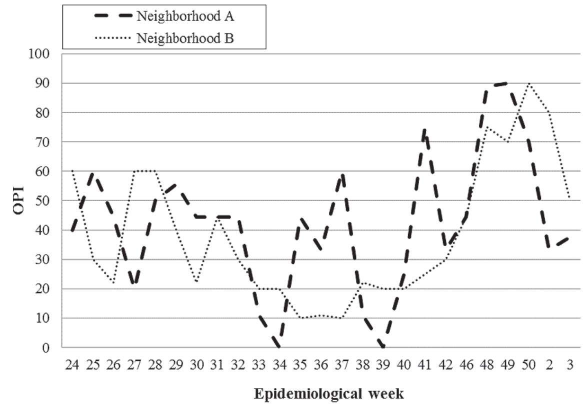

Figure 3: Ovitrap Positivity Index (OPI) in the two neighborhoods (A and B) depending on the week of the study.

The number of eggs found on the paddle was significantly higher than in the water ($p<0.05$), as expected. However, the percentage of eggs deposited in the water was higher than the 3% reported in the literature (5) in 96% of the samples, and reached up to 50% of eggs week 30 of the study (Figure 5).

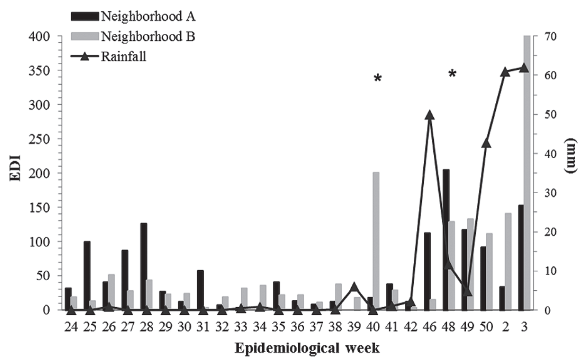

Figure 4. Rainfall and egg density index (EDI) in neighborhoods A and B, depending on the week of the study.

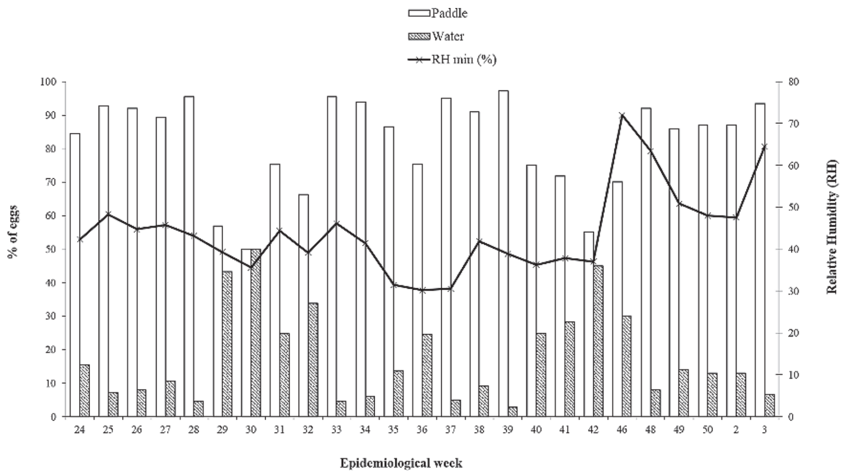

Figure 5. Minimum relative humidity and percentage of the eggs deposited in the paddle and water, depending on the week of the study.

In all, 2,098 (11.2%) eggs were found in the water. It was found that the weekly percentage of eggs in the water ranged from 4.56 to 50%. Another surprising result was that in 23 of the 500 surveys (20 traps * 25 weeks) eggs were found only in the water of the ovitrap. The percentage of eggs laid in the water was significantly higher (p=0.032) in the weeks where the minimum RH was less than 40%, as shown in Figure 6.

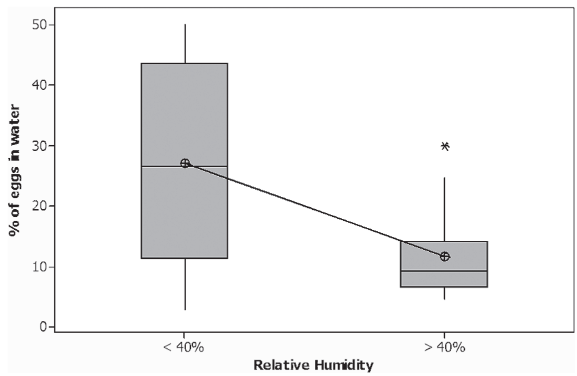

Figure 6. Boxplot of the number of eggs deposited in the water when the weekly minimum relative humidity was below and above 40%.

There was no relationship found between the amount of eggs deposited in the water and the other climatic variables evaluated.

In 53.6% of the positive traps, there were less than 30 eggs.

DISCUSSION

The ovitraps, which were handcrafted with disposable materials, presented excellent results and were sensitive to the detection of the *A. aegypti* populations, even in periods of drought, when populations are reduced. The results demonstrate that this kind of trap is still a good choice for the detection and monitoring of *A. aegypti* populations and can also be used in behavioral studies. The literature describes that the ovitrap is the most sensitive and cost effective method for detecting the presence of *A. aegypti*, in situations where population density is low, and it has proven to be effective in studies of the spread of the vector and of resistance to insecticides (3, 15, 21). Besides these advantages, the trap made in the present work from polyethylene (PET) bottles, still has strong economic and ecological appeal, since it re-uses material that would probably be discarded in the trash.

The similarities found in the studied parameters (number of eggs, proportion in the water and on the paddle) in the different neighborhoods indicates a certain homogeneity in the population of *A. aegypti* existing in the city of Salinas. The distance between the two neighborhoods is approximately 1000 m in a straight line, as shown in Figure 1. The literature describes that the ability of *A. aegypti* to spread by flight may reach 800 meters (15, 25) or even surpass 1.000 meters (19, 30). Moreover, periods of drought and low availability of breeding sites may increase the dispersion of females and hence gene flow between populations (9, 11). Therefore, the homogeneity observed between the two neighborhoods in Salinas can be explained by the small size of the city and predominantly dry climate, which would facilitate the spread of the insects between the two neighborhoods. Further studies should be performed to test their genetic homogeneity.

The large variation found among the OPI and EDI (0 - 90% and 0 - 1196, respectively) indicate that the population of this vector shows high levels of fluctuation between neighborhoods and the weeks of collection, as demonstrated in other studies (1, 23). This data can strengthen the evidence of mosquito migration between neighborhoods. In addition, from week 39, there was a growing trend of EDI and OPI, which can be explained by the onset of the rainy season in the area (Figures 3 and 4).

Some authors (1, 23) also found a positive correlation between the number of eggs collected in the ovitrap and climatic factors, especially rainfall. However, the correlation indexes observed in Cuiabá (23) did not exceed 0.217, which shows that the climatic factors seem to have more influence over the oviposition in Salinas than in Cuiabá, where the experiments were conducted. Because of this, it is recommended that the authorities of the north area of Minas Gerais reinforce entomological surveillance in the periods of the year in which the highest rates of rainfall and relative humidity are recorded. In addition, more detailed studies in neighborhood A are needed to determine which factors may be influencing the OPI in a more significant way.

The total amount of eggs found in water (11.2%) was almost four times higher than that described by other authors (5), who have considered the amount negligible. Other studies also found results different from these authors. A study with the use of filter paper as a substrate for oviposition, found 7.3% of the *A. aegypti* eggs in the water (26), while in a study conducted in Brazil, this ratio reached 8% (27). These data show that the amount of eggs that *A. aegypti* mosquitoes deposited directly in the water can be higher than those described in the literature, at least in some populations.

Our results also suggest that the deposition of eggs in the water is directly related to climatic factors, especially the relative humidity, in which a reduction below 40% appeared to be a stimulus for an increased deposition of eggs in the water (Fig. 6). Similar results were observed by other authors who found 9.3% and 12.6% of eggs in the water, in the rainy and dry seasons, respectively (7). However, the authors reported that the absence of a well-defined dry season prevented more accurate analysis. In an experiment with controlled laboratory conditions, some investigators also found large proportions of eggs in the water (20). The authors tested two mosquito populations (L and B) under conditions of high humidity (80%) and low humidity (51%). The percentage of eggs in water, in high humidity was 42.9 and 57.3% for L and B, respectively, and in low humidity conditions the numbers increased to 61.4% and 62.3%. In the present study, an increase was also observed in the percentage of eggs in the water at lower relative humidities.

These results are interesting because they may indicate a change in the behavior of *A. aegypti* females in response to drought. During these periods, the deposition of eggs directly in the water may ensure the continuity of the population, since the rainfall is reduced in these periods, which would prevent the hatching of eggs laid above the water surface. In addition, this deposition may accelerate the life cycle of the vector, since the eggs would hatch quickly, as well as protecting them against predation by ants and cockroaches (20).

However, to draw this conclusion we must assume two conditions: 1) the eggs found in the water did not fall off the paddle. We believe in this assumption because the paddle is an extremely rough and attractive substrate to mosquitoes (7, 26). Its roughness, together with the presence of sticky substances in the eggshell (sticky chorionic pads), would reduce the effect of fall of the eggs (8). Furthermore, it would be difficult to explain why the eggs fall more in the dry season than in the rainy season, when there are much larger populations of *A. aegypti* and eggs on the paddles. 2) The eggs in the water would need to have viability similar to the substrate. Unfortunately, we did not consider this during our analyses. Although some authors (27) have found low viability (only 2%), other authors have observed high rates of hatching among the eggs deposited in water - 47 to 53% (20) and 73 to 80% (28). Therefore we strongly believe that our results provide evidence to support the hypothesis that the oviposition in water can be a survival strategy of *A. aegypti* during periods of drought, at least in some populations, regions or climatic conditions.

It is important to say that, in addition to the ecological relevance, these results may have epidemiological importance, since the Ministry of Health in Brazil, recommends discarding the water or infusion in the ovitrap for the calculation of the infestation indices EDI and OPI (21). Thus, some of the eggs would not be counted, which could generate underestimated indices and ineffective actions to fight against dengue, especially in seasons with low relative humidity. Further studies must be performed to confirm these findings and provide more evidence.

The low number of eggs (<30) found in 56% of the ovitraps is evidence of the occurrence of skip-oviposition. It is known that *A. aegypti* females in oviposition activity can lay 60-120 eggs (8). Therefore, the low number of eggs found, indicates that females have not put all their offspring in the same breeding site. This behavior seems to be frequent in the city of Salinas. The importance of this behavior for the survival of the insects and for the spread of dengue is clear, since it avoids the loss of the entire reproductive investment of the females. Importantly, when the females go in search of breeding sites they also disperse the virus in the human population, as has been reported for this species in several studies (2, 6, 8, 13, 15, 24, 25). In Salinas, as elsewhere, the Skip-oviposition may be contributing to the behavioral homogeneity observed in the two neighborhoods and to the persistence of the vector throughout the year.

CONCLUSION

From these results, it can be concluded that the ovitrap manufactured from recyclable material may be a useful and environmentally friendly tool for monitoring and studying the behavior of *A. aegypti*. The deposition of eggs of this vector occurs homogeneously in the two neighborhoods tested, indicating a behavioral homogeneity in the population of the city. Climatic factors, especially rainfall and relative humidity have directly influenced entomological indices and the levels of mosquito infestation. The deposition of eggs in the water seems to be higher than that reported in the literature, especially in periods of low air humidity, which can influence the estimation of infestation rates by health agencies. Furthermore, skip-oviposition seems to be a frequent and important behavior in the survival of the insects in the semi-arid climate of Minas Gerais.

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