Uningá Review, v. 37, eURJ4418, 2022
doi.org/10.46311/2178-2571.37.eURJ4418
ISSN 2178-2571
Environment and Agrarian Sciences

Contribution of ovitraps in the control of the *Aedes aegypti* vector and reduction of dengue cases in the municipality of Ibirité in Minas Gerais

Contribuição das armadilhas ovitrampas no controle do vetor *Aedes aegypti* e redução dos casos de dengue no Município de Ibirité em Minas Gerais

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ABSTRACT
Controlling the vector population in the environment may contribute to reducing the clinical dengue cases. This study aimed to evaluate the use of ovitraps to monitor the entomological indices of *A. aegypti* and verify if the traps contribute to reducing dengue cases in Ibirité, MG. The traps were made using a medium plant pot, a wooden pallet, a clip and *Saccharomyces cerevisiae*. It was installed one trap every 15 days and, over three years (2019, 2020, 2021), resulted in 32 traps, distributed in the houses of 12 districts of Ibirité. After seven days of permanence, they were collected for eggs counting. A total of 60.433 eggs of *A. aegypti* were included in the Municipality, with the highest number observed in the Durval de Barros neighborhood. In 2019, 13.455 eggs were captured from January and May and between September and December. The IPO in the neighborhoods ranged from 9% to 54%, with the IDO ranging from 2 to 45 eggs. In 2020, 17.301 eggs were recorded, mainly from January to March and between October and December. The IPO in the neighborhoods ranged from 36% to 90% with the IDO ranging from 19 to 107 eggs. In the year 2021, there were 29,677 eggs between January and November that year, with the highest capture between January and March. The IPO was or higher for the three years of validity (70 to 10%), allowing us to infer that the traps help in the fight against dengue.
Keywords: *Aedes aegypti*. Arbovirus. Epidemiology.

RESUMO
Controlar a população de *Aedes aegypti* no ambiente pode contribuir para a redução dos casos clínicos da dengue. O objetivo deste estudo foi avaliar o uso das armadilhas ovitrampas para monitorar os índices entomológicos do mosquito *A. aegypti* e constatar se tais armadilhas contribuiriam para diminuir os casos de dengue em Ibirité, MG. As armadilhas foram confeccionadas utilizando vasos médios de planta, palheta de madeira, clip e *Saccharomyces cerevisiae*. Instalou-se uma armadilha a cada 15 dias e, ao longo de três anos (2019 a 2021), resultou em 32 armadilhas, distribuídas em residências de 12 bairros de Ibirité. Após sete dias de permanência, foram recolhidas para contagem dos ovos. Foram capturados 60.433 ovos de *A. aegypti* no Município, com o maior número observado no bairro Durval de Barros. Em 2019, foram capturados 13.455 ovos, principalmente de janeiro a maio e entre setembro e dezembro. O IPO nos bairros variou entre 9% e 54%, com o IDO entre 2 e 45 ovos. Em 2020, foram capturados 17.301 ovos, sobretudo de janeiro a março e de outubro a dezembro. O IPO nos bairros variou entre 36% e 90% com o IDO entre 19 e 107 ovos. No ano de 2021, foram 29.677 ovos entre janeiro a novembro daquele ano, com maior captura entre janeiro e março. O IPO foi alto para os três anos avaliados (70 a 100%), permitindo inferir que as armadilhas auxiliam no controle da população do vetor.
Palavras-chave: *Aedes aegypti*. Arbovirose. Epidemiologia.

Received: June, 22nd, 2022. Accepted: September, 22nd, 2022.

INTRODUCTION

Dengue is a viral disease caused by an arbovirus of the genus *Flavivirus* belonging to the family Flaviviridae (Costa, 2018) that presents four serotypes known in Brazil: DENV1, DENV2, DENV3 and DENV4, and also reported DENV5 found in the year 2013 in studies in Sarawak, one of the states of Malaysia, during serum analysis of patients isolated in 2007 (Mustafa, Rasotgi, Jain & Gupta, 2015). All serotypes can cause anything from an asymptomatic infection to the most severe forms, potentially leading to death (John & Rathore, 2019). Because cross-immunity to other DENV serotypes exists, subsequent infections increase the risk of developing dengue hemorrhagic fever, a severe (Sangkawibha, Rojanasuphot & Ahandrik, 1984; Pan American Health Organization [PAHO], 2021).

The main dengue vectors are the *Aedes aegypti* and *Aedes albopictus* mosquitoes, both responsible for the transmission of zika, chikungunya, Mayaro and dengue viruses (Chaves, 2018). In morphological analysis both mosquitoes have striped legs. The main dengue vectors are the *Aedes aegypti* and *Aedes albopictus* mosquitoes, both responsible for the transmission of zika, chikungunya, Mayaro and dengue viruses (Chaves, 2018). In morphological analysis both mosquitoes have striped legs (repetido). The *A. aegypti* mosquito has a lighter shade and the presence of a lyre-shaped design on the thorax (Forattini, 2002; Oswaldo Cruz Foundation [Fiocruz], 2008; Noronha, Campos & Cocco, 2017), while *A. albopictus* differs by presenting a longitudinal stripe on the back (Barros et al, 2021).

The *A. aegypti* found in tropical and subtropical regions the appropriate conditions for its development, establishing in Asia, Africa, Oceania and the Americas (Ribeiro et al., 2015). These conditions are the abiotic factors, the artificial and natural breeding grounds and important for the development of the larvae of both species (Riback, 2009).

A. albopictus is less prevalent in urban regions (Barros et al., 2021), being found more in the following regions: Asia, South America, Europe and North America (Kramer et al., 2015). In Brazil, *A. albopictus* is present in all regions, with prevalence in areas of dense vegetation, although this vector has also been found in areas that have been influenced by anthropogenic actions and urban areas with remaining forests (Carvalho, Lourenço-de-Oliveira & Braga, 2014; Heinisch et al., 2019; Rezende et al., 2020).

The breeding sites preferably used by *A. aegypti* and *A. albopictus* are represented by tires, cans, bottles, vase dishes, water tanks, barrels, among others, and the immature *A. albopictus* may have a preference for developing in bamboo hollows and tree holes (Oliveira & Biazotto, 2012). Among the artificial breeding sites, tires deserve special attention from zoonosis surveillance and control agencies. They are commonly discarded in vacant lots and in the rainy season are supplied with water that will act as breeding grounds for mosquitoes (Souza et al., 2008).

The transmission of viruses by mosquitoes can be influenced by the association of abiotic factors that correspond to temperature, climate changes and rainfall, relative humidity, and to biotic factors that relate to the immunity, genetics, and life expectancy of the disease vector (Adelman et al., 2013; Márquez Benítez, Cortés, Montenegro, García & Díaz, 2019).

A. aegypti has a seasonality that highlights the increase in temperature, rainfall and air humidity since rainy periods favor the breeding sites and consequently its development (Samuel, Adelman & Myles, 2016; Marinho et al., 2016; Fonseca et al., 2019). This mosquito has a peak activity in the twilight hours and is adapted to home environments due to its preference for artificial breeding sites and the hematophagy of the female for egg maturation (Fiocruz, 2008; Dias, Almeida, Haes, Mota & Roriz-Filho, 2010).

According to data from the Pan American Health Organization (PAHO, 2021), in the year 2013, more than 2 million cases of dengue were reported in the American continent with an incidence of 430.8 per 100,000 inhabitants. Among the cases, 37,692 severe cases and 1,280 deaths were reported in the continent. Already in 2019, just over 3.1 million cases, 28,000 severe and 1,534 deaths were reported. In Brazil, between the years 2008 and 2019, approximately 11.6 million cases of dengue, chikungunya and zika were reported. In this same period, 7,043 deaths from these diseases were confirmed. Dengue alone concentrated 91% of cases (10.6 million cases) and 91.2% of deaths (6,429 deaths), demonstrating the important burden of this arbovirosis compared to the others.

In this scenario, an ovitrap has been pointed out as an effective way to combat this endemic disease, since they are used to control the population of *A. aegypti* (Chiaravalloti et al., 2002; Jesus, 2018; Bantle, 2021). The use of these traps represents a low-cost alternative for monitoring the dengue vector in municipalities (Tulin et al., 2010). They provide a suitable environment for the female of *A. aegypti* to lay eggs and, after their quantification, they are destroyed, preventing the hatching of larvae.

Some authors point out that females can lay on average between 54.5 and 271.9 eggs during the first life cycle, with the female having a maximum number of up to 6 cycles recorded in the laboratory (Briegel et al., 2002; Beserra, Castro, J. W. Santos, Santos & Fernandes, 2006; Costa et al., 2010).

Given the above and taking into account the increase in the number of dengue cases and the use of alternative and low-cost methods, the objective of this study was to verify whether the use of ovitraps would be effective for the capture of *A. aegypti* eggs and its relationship with the reduction in the number of probable cases of the disease in the Municipality of Ibirité in Minas Gerais, in the years 2019 to 2021.

MATERIAL AND METHODS

Construction of the traps

The ovitraps were made by the Center for Epidemiological and Zoonosis Control of Ibirité (CCEZ) using a medium plant pot, a wooden reed in the shape of a glass laboratory slide measuring 26 mm wide and 76 mm long, a clip and a solution of *Saccharomyces cerevisiae* diluted in water. The *S. cerevisiae* yeast was inoculated into a 250 mL Erlenmeyer flask containing 50 mL of Sabouraud broth prepared according to the manufacturer's recommendation for 18 hours at 30°C. After the incubation period, the inoculum was transferred to 50 mL Falcons tubes and submitted to centrifugation for 15 minutes at 3500 rpm and 4 °C, after which the supernatant was removed and the yeast was made available for use in the traps. It is worth mentioning that the use of *S. cerevisiae* is an attractant for the mosquito females due to its use as a food source for the larvae, and the association relationship of this as a disperser of the yeasts in the environment (M. R. Barreto, Barreto & Anjos, 1998; Corrêa, 2013).

Figure 1. Ovitrap used.
Source: The authors.

To set up the trap, the straw was clipped to the transverse position of the vase and a part of it was immersed in a 300mL water solution with 6g of yeast so that the wood would be slightly moistened, favoring the deposition of mosquito eggs (Figure 1).

Area for the installation of the traps

The places to install the traps were previously defined by the CCEZ based on the data made available by the Municipal Health Department of Ibirité, of the neighborhoods that presented the highest records of dengue cases in the city. These neighborhoods were: Aparecida, Bela Vista, Colorado, Durval de Barros, Eldorado, Guanabara, Novo Eldorado, Palmares, Palmeiras, São José, Serra Dourada and Vila Ideal.

According to the guidelines of the Ministry of Health (Brazil, 2009), a trap should be placed every nine blocks or consider placing traps with a distance of 300 meters between them. The houses chosen were defined according to the profile of the most urbanized neighborhood and the availability of the resident to monitor the ovitrap.

The traps were installed in the household in order to reach the largest number of circulating females in search of blood. In all, 32 traps were installed in the 12 neighborhoods of the municipality described above. The first trap was set in January 2019 and the last one in November 2021 with analysis through biweekly cycles. However, in the year 2020, due to the SARS-COV-2 pandemic, between the months of April to September the traps were not installed due to social isolation, but were put back in October of that year and continued until 2021 with the appropriate analysis intervals.

The traps remained in the houses for 7 days and at the end of the seventh day they were collected and taken to the CCEZ, the wooden straws were removed and sent to the laboratory of the university for the quantity of eggs to be counted. The time frame for placing and removing the traps was determined to prevent the development of the mosquito to the adult stage, thus preventing the trap from becoming an artificial breeding site (Nogueira, Gushi, Miranda, Madeira & Ribolla, 2005). After counting the eggs, the straws were subsequently taken back to the CCEZ to be incinerated.

Egg counting and statistical analysis

For egg counting, a stereoscope microscope was used so that there was a significant increase in the visualization of the straws along with a manual numerical counter. In the observation of the straw, it was possible to verify the morphology of the eggs, black coloration, with a shiny aspect, tapered ends, bilateral symmetry and flattened surface (Pombo, 2016). At the time of spawning, the eggs of *A. aegypti* present a whitish coloration and later, after contact with oxygen, change to a shiny dark brown in which elongated designs can also be observed with the ends narrower than the center and an average size between 0.6 and 0.7mm (Barros et al., 2021).

The eggs collected in the ovitraps were analyzed by biweekly sampling cycles in 12 Ibirité neighborhoods, which, added together, allowed the calculation of the total number of eggs, in addition to the indices:

a) Ovitrap Positivity Index (IPO)

$$IPO = NAP/NAE \times 100$$

In which, NAP is the number of positive traps and NAE is the number of traps examined.

b) Eggs Density Index (IDO)

IDO = NO/NAP

In which, NO is the number of eggs and NAP is the number of positive traps.

c) Vector Density Index (IDV)

IDV = NO/NAV

In which, NO is the number of eggs and NAV is the number of inspected traps.

For the analysis of the quantitative data obtained, were used Excel software and GraphPad Prism 7. For the comparison of the grouped data, the Wilcoxon and One-Way Anova T Test were used to determine if there was a significant difference between the data.

RESULTS AND DISCUSSION

During the development of this study, only *A. aegypti* eggs were found, whose smooth symmetry can be seen in Figure 2, and no eggs of *A. albopictus* were found, which, in turn, have an average of 1 mm and appear with a striated surface, with small, grain-like projections around the structure, as reported by Barros et al. (2021).

Figure 2. Eggs of *A.aegypti* observed in a pallet by microscopy.
Source: The authors.

The total number of eggs captured in the traps in the year 2019 was 13.455, in 2020, 17.301 eggs were captured, in 2021 there were 29.677 eggs. In the year 2020, even with the interruption in the placement of the traps in the month of April caused by social distancing and the return of the traps in October of that year, the high number of captured eggs could be observed.

This may be related to the preference of females for ovitraps rather than other breeding sites, since the former have a higher concentration of *S. cerevisiae* that will serve as food and benefit the development of larvae (Souza et al., 2016). In studies developed at the Instituto Oswaldo Cruz (IOC/Fiocruz), it was pointed out a nutritional advantage in the use of *S. cerevisiae* for feeding *A. aegypti* since the larvae and adult male mosquitoes gained approximately twice the weight, in addition, the research also pointed, for the first time, the discovery and the action of the enzyme beta-1,3-glucanase in the degradation of the cell wall of yeast cells allowing the larvae to absorb their nutrients (Souza et al., 2016).

When analyzed the number of eggs captured in the traps and correlated with the probable dengue cases reported in the three years of the study, it is possible to see that as the number of eggs captured in the traps increases, there is a reduction in the probable dengue cases reported by the Minas Gerais Department of Health (Figure 3).

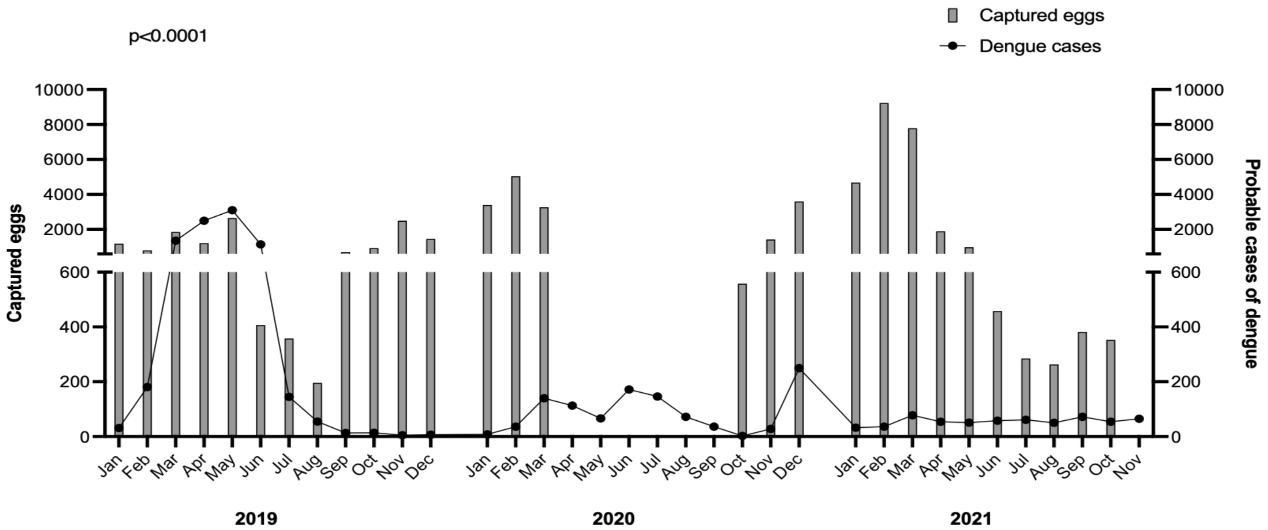

Figure 3. Number of captured eggs in traps and dengue probable cases notified in Ibirité, MG. The dates were studied for three years (2019, 2020, 2021).
Source: The authors.

In 2019 (Figure 3), the months of March to May had the highest number of eggs captured and it can be seen that egg capture follows the peak of probable dengue cases in the municipality, indirectly reflecting that the ovitraps contributed to the vector control circulating in the municipality that year, where the significant increase in the number of dengue cases was expected due to 2019 being a dengue epidemic year.

In the year of 2020 (Figure 3), there was greater capture of eggs between January and March, which directly reflected a lower number of probable cases of dengue compared to the same period of the previous year. With the return of rainfall since October, it was possible to observe an increase in the number of eggs captured, as well as Miyazaki et al. (2009) who identified a positive correlation between the number of eggs and volumetric precipitation. Regis et al. (2008) state that rainy and hot periods are responsible for the increase in female density and proliferation of the mosquito when compared to dry and hot periods. Even so, evidence in the literature shows that there is still a significant density of the vector in the period of low rainfall and, therefore, the actions to combat the vector and monitoring should not be interrupted because they can prevent the eggs laid by females from hatching and finding favorable conditions for dissemination of the virus in the population (Luz et al., 2020).

When observed the data from the year 2021 (Figure 3), there was a high rate of egg capture, only between the months of January and May in that year 19,901 eggs were captured, which may have occurred due to the rainy season as corroborated in the studies of Marinho *et al.* (2016). In the months from April to May 2021, the traps, even during the dry season, continued to be effective in capturing eggs, and despite the return of rain between October and November, there was a reduction in the rate of egg capture and in the notification of probable dengue cases in the municipality (Figure 3). Between January and December 2021, about 29,677 eggs of *A. aegypti* were captured and when compared to the numbers of probable dengue cases, which were 563, there was a reduction in the number of cases compared to 2019 with about 8,546 probable cases (Figure 3).

The indices obtained by the analyses of the ovitraps can demonstrate the following phases for monitoring the *A. aegypti* mosquito: 1) control: when the risk of transmission is very low (IPO less than or equal to 40%); 2) alert: the IPO indices are between 41 to 61%; 3) risk: when the IPO and IDO are above 60% or 60 eggs according to the studies of Costa, Avendanha, Leite, Reis and Lisboa (2007). In 2019, IPO in the neighborhoods ranged between 9 and 54% with IDO pointing between 2 and 45 eggs, these data put the neighborhoods in an alert phase for monitoring and control (Figure 4, A and B).

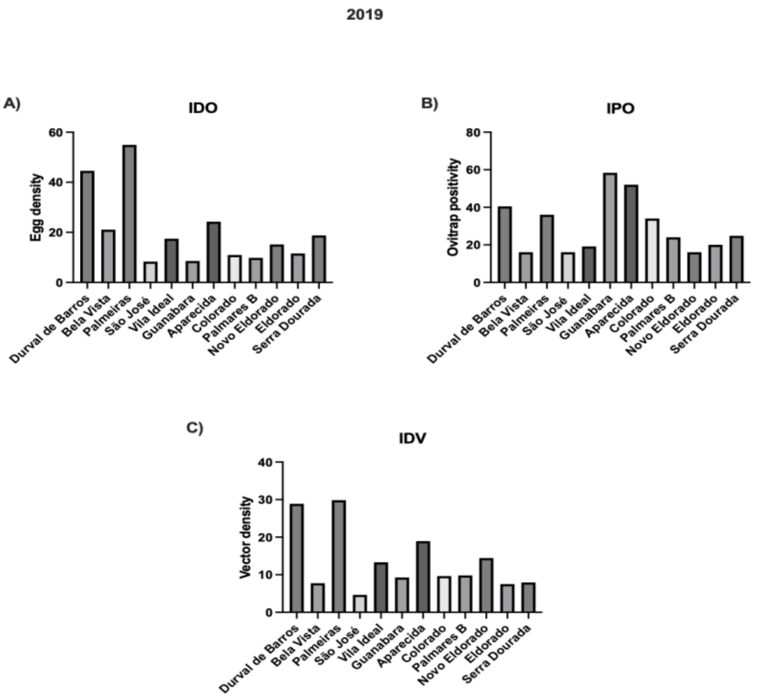

Figure 4. Index sampled in the year 2019. A) Eggs Density Index (IDO). B) Vector Density Index (IDV). C) Ovitrap positivity Index in percentage (IPO).
Source: The authors.

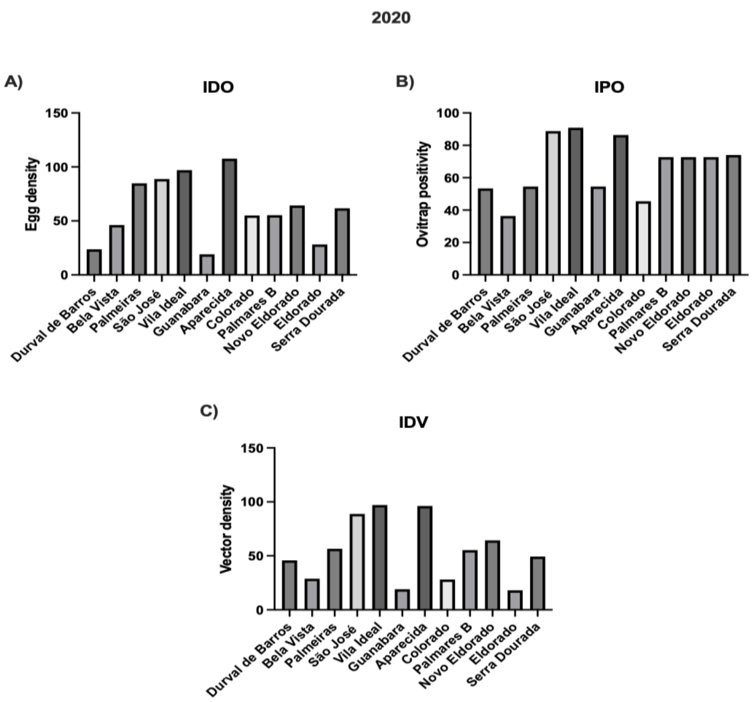

Figure 5. Index sampled in 2020. A) Eggs Density Index (IDO). B) Vector Density Index (IDV). C) Ovitraps Positivity Index in percentage (IPO).
Source: The authors.

In 2020, the IPO in neighborhoods varied between 36% and 90% with the IDO that varied between 19% and 107% (Figure 5, graphs A and B). Zequi, Oliveira, Santos and Lopes (2018), found the IPO oscillating between 9,8 and 100% and the IDO between 5,17 and 87,2 eggs on average, and this points to the sensitivity of the trap even in low or high infestation of the mosquito.

In 2021, the IPO was the highest for the three years evaluated, and it showed the neighborhoods with the highest number of reported dengue cases, as the highest between 70 and 100% (Figure 6, graph B).

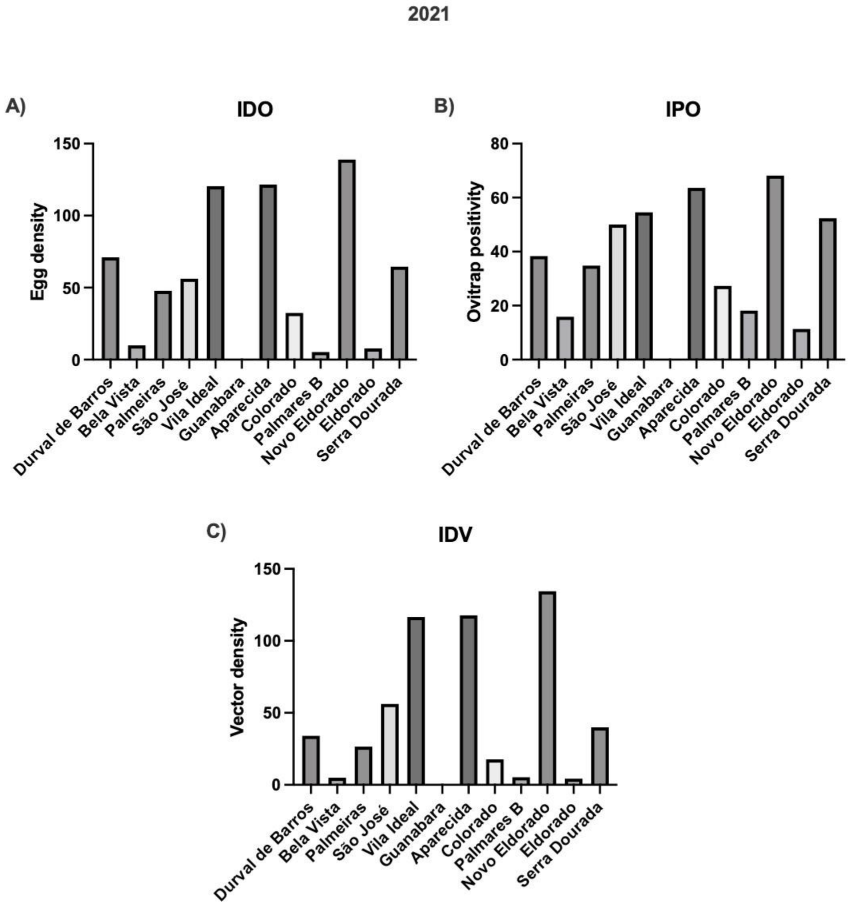

Figure 6. Index sampled in the year 2021. A) Eggs Density Index (IDO); B) Vector Density Index (IDV); C) Ovitraps Positivity Index (IPO) in percentage.
Source: The authors.

This can be affirmed because the location of the traps is chosen according to the streets with the highest number of dengue cases, evaluated monthly by CCEZ. The IDO in the months of heaviest rainfall reached values greater than 200 eggs, indicating that the neighborhoods evaluated represent a high risk of infection of the inhabitants by arboviruses transmitted by *A. aegypti*.

By observing and analyzing the indicators provided by the ovitraps, it was observed that the IDV and IDO showed similar behavior in the neighborhoods evaluated between 2019 and 2021 (Figures 4, 5 and 6, graphs A and C). The IDV of the Durval de Barros neighborhood, which had the highest sampled rates, ranged between 0.8 and 222.6 in 2019; between 45 and 186.5 in 2020 and between 11 and 1,357 in 2021 (Figures 4, 5 and 6, C graphs), with the highest rates in periods of high humidity and heat and the lowest rates observed in the driest months between April and July of each year, as corroborated in other studies found in the literature (Regis et al., 2008; Luz et al., 2020).

The high percentage of eggs collected in the Durval de Barros neighborhood can be attributed to the fact that this is the most urbanized area of the municipality, also presenting a disorderly growth. Barreto, Resende, Eiras and Demarco (2020) claim that socio-environmental factors of settlement may contribute to the higher prevalence of *A. aegypti* eggs in these regions.

Some studies report that the IPO indicator equal to or greater than 40% positivity is an indicator of the risk of arbovirus transmission (Gomes, 1998; Barreto et al., 2020). In the present study, it was possible to observe that the neighborhoods with the highest number of reported cases of dengue and that had traps installed, had an IPO equal to or greater than 40% in the hottest and humid months and even in the driest and coldest months, some neighborhoods still had IPO above 40%, such as Durval de Barros, Novo Eldorado, Serra Dourada and Vila Ideal (Figures 4, 5 and 6, B graphs).

The traps placed remained positive throughout the period. This phenomenon of positivity can be associated with the behavior of *A. aegypti* females that distribute their eggs in different recipients and do not lay them all in a single breeding site, a phenomenon called oviposition in jumps (Domingo et al. 2005). The IPO data in the neighborhoods of Ibirité allowed to indicate that the areas studied are at high risk for transmission of dengue and other arboviruses by *A. aegypti*. The IDO and IDV by neighborhood oscillated similarly, pointing in most of the cycles, the amount of eggs superior to 100 eggs in the positive and inspected ovitraps, respectively. This observation of the IDO and IDV indicators can be attributed to the high rate of infestation by the mosquito, which was also observed in the studies by Barreto et al. (2020). It also correlates the positive effect of the rainy season on the analyzed indices.

This data is expected because in rainy periods and with higher temperatures, the female *Aedes* has a better ability to lay and feed. The temperature that most favors this mosquito varies between 24ºC and 28ºC and if added to high humidity, above 70%, *A. aegypti* reproduces with greater intensity, increases hematophagy activity and the efficiency of reproduction of the virus internally, its survival and oviposition (Ajuz & Vesterna, 2013). Therefore, the use of the oviposition trap would be an appropriate method of detecting the presence and the variation of the mosquito population density in the neighborhoods of Ibirité, allowing the monitoring of the increase of the vector and the definition of combat strategies in the places with the highest incidence of the mosquito. and dengue cases.

Data collected to date indicate that ovitraps can contribute to reducing the number of dengue cases in Ibirité. With the removal of eggs, the abundance of the vector decreases and, consequently, fewer mosquitoes could carry out their complete biological cycle. This is important information to help the Municipalities of the metropolitan region of Belo Horizonte in the fight against the vector and to understand the factors that have led to the greater number of mosquitoes in some monitored points compared to others. These data can serve as an instrument of regional observation to carry out improvements in sanitary structures, in garbage collection, in the monitoring of vacant land and allow interventions by the public authorities in the Municipalities that will use these traps.

The data also suggest that the ovitraps placed fortnightly interfere positively to reduce the number of probable cases of dengue, which can be observed over the coming years with the maintenance of the traps in the municipality. And this reinforces the importance of the work that has been carried out in Ibirité since January 2019 as a measure to monitor and at the same time combat the mosquito vector of dengue and other arboviruses.

CONCLUSION

The year of 2016 presented a high number of dengue cases in the population of Ibirité - MG. In 2019, the CCEZ started the placement of traps called ovitraps in a collaboration with UEMG - Ibirité unit that since then has been working for the monitoring of dengue cases in the municipality and their correlation with the capture of *A. aegypti* eggs in the traps since the placement of traps is defined according to the places that register cases of the disease.

The number of recorded dengue cases in 2019 was lower compared to the year 2016, and in contrast the number of eggs collected in the ovitraps increased between the years 2019 and 2021 showing IPO rates higher than 40% in most of the analyzed cycles and this reflected in the reduction in the number of dengue cases in 2019 of about 20% when compared to the year 2016, even with the increasing increase in the population of the municipality.

The data obtained suggest the importance of using ovitraps as a way to monitor dengue cases in the municipality and, at the same time, demonstrate that the use of such traps can contribute over the years to the reduction of cases of arbovirosis in Ibirité. Further studies should be done to seek explanations to justify the occurrence of dengue in specific and recurrent points in the municipality in order to assist in decision making by the public authorities. It is also important to consider that in order to combat the vectors of diseases, awareness raising actions in the area of education and neighborhood associations about the fight against arboviruses are of paramount importance, as they help in the proper management in the prevention and control of endemic diseases.

ACKNOWLEDGMENTS

To the Center for Epidemiological and Zoonosis Control of the Municipality of Ibirité for collaborating in this study by installing and setting the traps in the households. To the Program of Support to the UEMG Extension (PAEx) for funding the scholarships for undergraduates involved in this work.

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