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Peridomicillary peculiarities of rural communities in Goiás-Brazil, with families affected by arboviruses

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Abstract

Arboviral diseases such as dengue, Zika and chikungunya infect populations in countries with predominantly tropical and subtropical environments, where their vectors are adapted. This research aimed to identify potential favorable sites to *Aedes aegypti* reproduction at rural communities where dengue, Zika and chikungunya prevalence is high, geographically located in Goiás-Brazil, and to analyze the relationship between breeding sites quantity and these three arboviruses seroprevalence. In 137 families from 13 rural communities, information was obtained between October/2021 and November/2021. Data were analyzed quantitatively and qualitatively trough Pearson's correlation and Cluster analysis. 92.7% of the households, possessed, at least one potential mosquito breeding ground. Water containers, despite of being uncovered, retain water and create an ideal environment for mosquito's evolutionary cycle. Potential breeding grounds were observed related, mainly, to the lack or absence of precariousness of basic sanitation services. The community with the highest number of breeding grounds and suspected vectors of *Aedes aegypti* was also the one that resulted in the highest number of patients with dengue fever. The presence of places for *Aedes aegypti* development in the studied environment may be responsible for arboviruses spread, affecting a significant part of the population. Therefore, the present study might warn municipal health authorities for implementing public policies such universal sanitation, as a measure that indirectly provides vector control especially *Aedes aegypti*, when it also proliferates in rural areas, risking the population health.

Introduction

Aedes aegypti (Diptera, Culicidae) is a mosquito that transmits multiple arboviruses, infecting populations in countries with predominantly tropical and subtropical environments (Who 2019). Its dissemination is, mainly, influenced by environmental conditions (climate typology, altitude) and its life cycle with four distinct stages (egg, larvae, pupa and adult form) (Zara et al. 2016; Fachinelli, Badolo and McCall 2023), according the quantity of existing larvae on the same breeding spot, local temperature and food availability (Who 2019). Therefore, aiming to reduce its proliferation, become necessary the interruption of its life cycle, through breeding spots elimination (Who 2019), which generally can be found in human dwelling surroundings (Chandrasegaran et al. 2020; Ouédraogo, Toé and Sombié 2022). In 1956, *Aedes aegypti* was eliminated from Brazil, but reintroduced in the 70's (Csillag 2000). Since then, the mosquito population has been increasing, reaching all Brazilian states (Silva and Machado 2018) and, consequently, humans from all regions are being infected by any arbovirus like dengue (SDENV-1-4), Zika (ZIKV) and chikungunya (CHIKV), where Midwest region is leading to positive cases, with high dengue incidence in the last two years (Brazil 2022).

The presence of mosquitoes belonging to the genus *Aedes* (Stegomyia), specifically the species *Aedes aegypti* (Linnaeus, 1762) is not only detected in urban areas, but in rural areas across various regions worldwide, where arboviruses such as dengue, Zika and chikungunya generally propagate, as evidenced in the literature, over the years (Harrington et al. 2014; Olano 2016; Cauich-Kumul et al. 2018; Silva and Scalize 2023), This prevalence is particularly significant in countries characterized by tropical and subtropical climates, such as Brazil (Barbosa et al. 2009; Saraiva et al. 2013; Stahlöfer, Borsekowsky and Müller 2021; Almeida, Cota and Rodrigues 2020). Although arboviral diseases mainly affect urban population, driven principally by unplanned urbanization (Almeida, Cota and Rodrigues 2020), combined with socioecological factors (Kolimenakis et al. 2021), *Aedes aegypti* or *Aedes albopictus* carrying arboviruses have been found in rural areas around the world, including Brazil (Pérez-Castro et al. 2016; Ricas-Rezende et al. 2020), with relevant seroprevalence rates (Silva-Nunes et al. 2008; Anish, Vijayakumar and Leela-Itty-Amma 2011; Kumar et al. 2020), raising the following question: are arboviruses spreading locally in rural area, or are they imported from urban locations? However, *Aedes aegypti* is opportunely adapted to countries like Brazil, where socio-environmental conditions favor its proliferation and dissemination (Li et al. 2021; Silva 2022; Oliveira et al. 2023), demonstrating that human rural populations, may not be free of the diseases transmitted by these vectors.

In this context, according to Health Surveillance Secretary of the Ministry of Health of Brazil, there is a crucial necessity to intensify *Aedes aegypti* breeding sites control, in order to avoid greater arboviruses transmission, each year (Brazil 2022). Thus, World Health Organization (WHO) recommends breeding sites identification and elimination, at household level, as potential places for *Aedes aegypti* proliferation, as well improving logistics of solid wastes improperly handled and irregular water storage, factors contributing to arboviruses spread (Cavalcanti, Oliveira and Alencar 2016; Kampago et al. 2021; Tezzo et al. 2021). Therefore, apparently harmless items such as bottle caps, small cans and plastic cups that can be found backyards, with water accumulation, become adequate breeding sites for development of different mosquitoes species (Kumar et al. 2020; Mol et al. 2020; Cabrera et al. 2022).

Thus, the objective of the present work was to highlight specific places that may favor *Aedes aegypti* reproduction, located in peridomicile of rural communities in Goiás, with families affected by dengue, Zika and/or chikungunya; as well to analyze the relationship between breeding sites quantity and seroprevalence rates for the three arboviruses.

Materials and Methods

The study was performed in 13 rural communities, distributed in 12 municipalities in Brazilian state of Goiás (Fig. 1), within the scope of the project called Saneamento e Saúde Ambiental em Comunidades Rurais e Tradicionais de Goiás (SanRural).

In these communities, peridomicile peculiarities (primary data) and human population seroprevalence for arboviruses (secondary data) were analyzed. Both data come from the SanRural project and were collected in the same households, in different periods. Figure 2 shows a flowchart of the research development, which are described in the subsequent items.

With respect to the spatial distribution of rural communities based on the nearest urban area; the shortest road access distance between each rural community and the nearest urban center were obtained using vector files, in shapefile format, for municipal urban perimeters, all available in the Geoinformation State System (GSS) (Sieg 2014), considering urban expansions presence, aided with Google Satellite images. In the same way, distances between rural communities and capital of the state of Goiás, Goiânia were obtained, where laboratorial analyzes were developed.

Figure 1 Rural communities' location and their respective municipalities in Goiás, Brazil. Its capital Goiânia is highlighted in black, where the laboratory analysis was developed. Source: Created by authors. Note: Community/Municipality: Canabrava/Flores de Goiás = 1; Extrema/laciara = 2; Itajá Il/Goianésia = 3; João de Deus/Silvânia = 4; Lageado/São Miguel do Araguaia = 5; Landi/Santa Rita do Novo Destino = 6; Pelotas/Monte Alegre de Goiás = 7; Rochedo/Professor Jamil = 8; Fazenda Santo Antônio da Laguna/Barro Alto = 9; São Lourenço/Uruaçu = 10; São Sebastião da Gargan-ta/Silvânia = 11; Sumidouro/Padre Bernardo = 12; Taquarussu/Campos Belos = 13.

2.1 Peridomicile peculiares

Between October/2021 and December/2021, the most relevant peridomicile characteristics were obtained through in situ data collection. Sampling design consisted in register families considered as primary sampling units (PSU) whose members were residents with fixed residence in a portion (Zone or area) of the community, and were present or temporarily absent during the activities of this research. Thus, each PSU were selected by systematic random sampling method. On stage one, considering family members (family members over 18 years of age) and with a responsible for the household, in agreement with the rest of this family. The project was approved by the Research Ethics Committee of the Federal University of Goiás (UFG), CAAE n° 87784318.2.0000.5083.

In these 13 rural communities, a were found 421 households, with an estimated population of 1263 inhabitants. From them, 137 households were sampled (35,2%) with 411 people living in them, in average of 3.0 inhabitants/household. Data from each selected household was collected with the aid of a survey, consisting of an observational checklist considering geographic coordinates (latitude and longitude), images of potential mosquito growing places (photographs) and, mainly, presence or absence of Culicidae breeding sites per household (PPBSH) and per community (PPBSC), registering the occurrence of breeding sites with existence, or not of suspicious (larvae, pupae and/or adult) of *Aedes aegypti* (BSSAaegypti).

Regarding the identification of *Aedes* (Stegomyia) vector, specifically *Aedes aegypti* species (Linnaeus, 1762), based on scientific evidence of the main breeding sites facili-tating the proliferation of this mosquito species, various life cycle stages were observed. These stages were considered possible *Aedes aegypti* vector because of its specific attributes that significantly differ from those of other mosquitoes. A meticulous on-site analysis was conducted, including egg types, larvae, and the adult form examination. This culicid typology can be observed in diurnal mosquito species with black coloration, white stripes and spots. It belongs to Arthropoda phylum (jointed appendages) from Hexapoda class (three pairs of legs), within Diptera order with one (anterior) pair of functional wings and other, posterior, pair transformed into halters belonging to Culicidae family and the *Aedes* genus (Paho 1986).

2.2 Arboviruses seroprevalence

Seroprevalence data for arboviruses as SDENV-1-4 (SD), ZIKV (SZ) and CHIKV (SC), and their combination (SD_Z_C), were obtained from Pagotto et al (2022), confirmed through laboratory test with blood samples collected from 272 inhabitants (21.3%) living in 193 households (more than the 137 households visited for peridomicile inspection, due to the absence or refusal of the peridomestic investigation). On the other hand, blood samples were collected from, at least, one inhabitant in some households. Briefly, a 5 mL blood sample was collected by venipuncture using disposable syringes and needles.

Therefore, these samples were carried to laboratory for being processed. First of all, each sample were centrifuged and, subsequently, serum plasma was aliquoted in properly identified cryotubes, packed in nitrogen cylinders and sent to Virology and Cell Culture Laboratory of the Tropical Pathology and Public Health Institute at Federal University of Goiás (LabViCC/IPTSP/UFG). For serological investigation, Immunoenzymatic Assay (EIE) was applied to serum samples, and subjected to IgG class specific antibodies for dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) viruses, using commercial kits and following manufacturer's instructions: Euroimmun Zika virus IgG; Euroimmun dengue virus IgG; Euroimmun chikungunya virus IgG. Seroprevalence rates (%) for dengue, Zika and chikungunya by rural community were obtained by dividing the number of residents with a positive IgG for each arbovirus, by the number of residents who had blood drawn (Pagotto et al. 2022). The study area was the same as the sample design in which peridomicile peculiarities were registered. Both data are part of the SanRural Project.

2.3 Statistical analysis

Statistical process began with descriptive statistics, as basis for multivariate analysis. Subsequently, Pearson's correlation analysis was applied for each community where variables with zero standard deviation were excluded from correlation matrix. The hypothesis tested here was also applied to verify if correlations are significantly, different from zero, using three statistical significance levels as used in literature, being 1%, 5% and 10%. In a second stage, cluster analysis was developed, aiming to classify 13 rural communities into groups, according to their characteristics in terms of demographic (households/km²), geographic (distance between households), seroprevalence for the three arboviral diseases and the number of breeding sites favorable to *Aedes aegypti* reproduction.

Hierarchical Cluster Analysis is an unsupervised classification, which hierarchizes objects into different groups containing, each one, their peers according to a statistical distance function (Flynn, Jain and Murty 1999). One of the ways to obtain this similarity measure is using Euclidean distance (D) (Gauch 1982), as used to verify relationship between dengue and socio-environmental variables in Northeast Brazil (Silva and Machado 2018). Hierarchical clustering analysis results can be represented graphically by a dendrogram (or tree diagram) representing the organization of certain factors and variables, which demonstrate clusters produced by hierarchical grouping. In this graphic, the Y axis represents the objects, while X axis rep-resents Euclidean distance, or similarity level, between clusters (Bibby and Mardia 1979) that is represented, in the present study, trough Ward's method. This methodology tends to minimize sum of squares between two groups in relation to all variables. Finally, the definition of ideal number of clusters was performed by Elbow method.

To apply cluster analysis, raw data were grouped by community, so that binary variables are represented by the proportion of the characteristic of interest in the com-munity, seroprevalence for dengue, Zika and chikungunya (SD, SZ and SC, respectively), presence of possible breeding sites by households

(PPBSH) and places with suspected *Aedes aegypti* (BSSAaegypti) and the variable presence of possible breeding sites by community (PPBSC), represented by the average amount in the community. In the assessment of community clustering, the following variables were considered: SD, SC, PPBSC and SD_Z_C (seroprevalence for dengue, Zika and/or chikungunya). The average distances between all community households and population density were obtained in recent study (Pinheiro et al. 2022).

Results

Figure 3 illustrates households communities visits, applying a survey to the house-hold leader (Fig. 3a), as well as cataloging breeding sites around the property (Figs. 3b to 3f), which are favorable to Culicidae proliferation.

The breeding sites found in the peridomestic environments (Fig. 3) were similar to those reported in the literature when associated with basic sanitation (Table 1). Tire presence represented 26% of the total frequency, as the most frequent container, followed by plastic drums (22%), and water tank and animal water troughs, both with 14%. These results corroborate Ngugi et al (2017) found, who reported that the most frequent types of habitat were buckets, drums, tires and pots, representing more than 75% of all pupae, concluding that efforts to control Culicidae population could be directed towards eliminating these breeding sites, being a way cost-effective way to reduce arboviral transmission.

Table 1

Observed in thi <i>situ</i>)	s research (<i>in</i>	Literature data							
Local	Occurrence (%)	Local ¹	Reference						
Plastic drums	22.0	Large reservoirs that are poorly or uncovered: water tanks, gallons, casks.	(Guagliardo et al. 2014; Ngugi et al. 2017; IOC/Fiocruz 2017; Rahman et al. 2021)						
Water tank (Fig. 3b)	14.0	Casks.							
Bucket	12.0								
Can	2.0								
Animal water troughs (Fig. 3d)	14.0	Containers for watering animals, among others	(Brazil 2013; Dalpadado, Amarasinghe and Gunathilaka 2022)						
Tire (Fig. 3e)	26.0	Accumulated and/or scattered	(Lopes et al. 2004; Troyes, Vellegas and Troyes 2006; Manrique-Saide et al. 2008; Ngugi et al. 2017; IOC/Fiocruz 2017; Dieng et al. 2018; Silva et al. 2020; Rahman et						
Pan with rain water	4.0	solid waste; organic waste outdoors.	al. 2021)						
Bottle and scattered waste (Fig. 3c)	2.0								
Rudimentary well (Fig. 3f)	4.0	Rudimentary wells, poorly or uncovered.	(Barrera et al. 2008; Mackay et al. 2009; Somers et al. 2011)						
Total	100.0								

Complementing Fig. 3 and Table 1, Fig. 4 illustrates the breeding sites with their percentages found in the present research. Among breeding sites, tire is an important container for Culicidae oviposition. This was evidenced in the present research, where immature individuals of suspected *Aedes* were observed, mainly in discarded tires (26.0%), corroborating the literature.

In this context, Lopes et al (2004) aiming to analyze *Aedes aegypti* and *Aedes albopictus* spatial dispersion between rural and urban areas in Paraná (Brazil), verified that tires contain the largest amounts of Culicidae larvae, and both were dispersed in rural area, and recommend resizing control areas, as rural communities can be refuge-like for these vectors. Additionally, other variety of water containers (Bucket – 12.0%; Can – 2.0%; Pan with rain water – 4.0%; Bottle and scattered waste – 20% and Rudimentary well – 4.0%) appear as potential *Aedes* breeding sites, contributing to its greater or lesser spread in rural and urban areas. Besides that, environment influences *Aedes aegypti* and *Aedes albopictus* abundance, with *Aedes albopictus* being predominant in rural areas in relation to *Aedes aegypti* (Braks et al. 2003). However, contrary results can be found in literature (Djiappi-Tchamen et al. 2021). Ricas-Rezende (2020) observation is considered the first report of *Aedes albopictus* infected by dengue and Zika viruses in Brazilian rural areas, specifically in Espírito Santo state. The population of this vector is growing in the Americas (Garcia-Rejon et al. 2021) and is present, already, in all Brazilian states, with emphasis on the Southeast and Midwest regions (Variza et al. 2022).

In Brazilian Midwest, especially in the rural communities studied in the present work, the containers are more susceptible to prolonged water storage due to rain frequency, combined with the lack of proper handling of disposable debris by residents. Dalpadado, Amarasinghe and Gunathilaka (2022) reported that

artificial breeding sites represent 80% of the places found with *Aedes* larvae. Kroth et al (2019) evidenced Culicidae strong preference for oviposition in outdoor containers, with preference of those with higher organic matter concentrations and, according to the authors, it helps to a better understanding of *Aedes aegypti* for in-habiting anthropogenic environments, being possible to verify by nutrients availability analysis and breeding sites.

The contact of *Aedes aegypti* eggs with water is the fundamental stimulus for its development cycle (Silva et al. 1993). Basic sanitation absence or precariousness allows all these arboviruses vectors proliferation (Almeida, Cota and Rodrigues 2020), which was observed in the analyzed communities here, in greater or lesser quantities. According to residents, the main motivation for storing water, for different reasons and long-term use, is due to difficulties of access to it. Other Culicidae breeding sites are reported in the literature, such as rain drains and manholes (Paploski et al. 2016); however, they were not identified in the present study, due to the lack of basic sanitation facilities.

Table 2 presents a summary of peridomiciliary aspects, obtained from supplementary material, and seroprevalence data for SDENV-1-4, ZIKV and CHIKV, indicating positivity for immunoglobulin G tests (IgG class) antibodies presence, against these agents. Among the 137 households visited, the present research found the average of 3.0 inhabitant/household. In in 46.7% (64/137) of them (Table 2), at least one person had already been infected at some point in their life with one of the serotypes of SDENV-1-4, ZIKV and/or CHIKV, corresponding to 21.7% (89/411) of the residents, where total seroprevalences were 29.0% for SDENV-1-4, 3.9% for ZIKV and 12.9% for CHIKV, in all 13 communities visited. These results showed similar behavior from lower to higher incidence found by Li et al (2021), where they observed prevalence of 38.0% for dengue, 18.0% for Zika and 25.0% for chikungunya.

However, with lower incidence of arboviruses, mainly Zika; Li et al (2021) in their meta-analysis, analyzed 133 papers with 176,001 participants, reporting rural and urban populations seroprevalences, from developed and developing countries, with similar values for dengue and chikungunya obtained in the present research. This support, also, the hypothesis that arbovirus transmission can occur in rural locations as well as in urban areas, especially when there are favorable environmental conditions for vector proliferation.

Table 2 Breeding sites quantity with and without suspicious presence of *Aedes aegypti* forms and positivity for dengue (SDENV-1-4), Zika (ZIKV) and chikungunya (CHIKV), with respective seroprevalence, in rural communities, Goiás, Brazil

Community	Visite house	d sholds	Breeding sites with suspicious		Favorable places for		Positive for DENV,		Seroprevalence ^(d)									
			<i>Aedes aegypti</i> forms		<i>Aedes</i> reproduction		ZIKV and/or CHIKV		for SDENV-1-4, ZIKV e CHIKV at community level									
	n°		n°		%	n°		n°		%								
	ID	Res	Y	Q ^(a)	ID ^(b)	Y	Q ^(c)	ID	Res	SDENV- 1-4	UL	IL	ZIKV	UL	IL	CHIKV	UL	IL
1	8	24	1	3	12.5	8	25	5	7	49.0	26.0	72.4	0.0	0.0	0.0	17.9	6.9	39.1
2	10	30	6	8	60.0	8	32	9	17	82.4	59.0	93.8	5.9	0.7	35.2	4.2	0.9	18.4
3	11	33	2	2	18.2	8	20	2	3	11.4	2.7	37.1	0.0	0.0	0.0	11.4	2.7	37.1
4	7	21	1	2	14.3	7	16	1	1	0.0	0.0	0.0	0.0	0.0	0.0	20.8	3.9	62.8
5	13	39	3	3	23.1	11	44	8	12	64.6	33.6	86.8	24.9	4.5	70.1	14.2	2.7	49.4
6	6	18	0	0	0.0	6	23	5	6	63.7	29.8	87.9	23.4	3.9	69.8	12.3	2.9	40.0
7	10	30	6	6	60.0	10	38	2	3	6.4	1.3	26.2	0.0	0.0	0.0	2.6	0.3	19.4
8	19	57	6	6	31.6	19	83	7	7	24.4	7.6	55.7	0.0	0.0	0.0	21.8	6.5	52.5
9	9	27	2	5	22.2	9	49	6	11	45.2	23.6	68.7	4.1	0.7	21.3	14.1	5.2	37.0
10	11	33	4	4	36.4	10	25	8	11	49.4	27.0	72.1	24.9	8.5	54.1	26.6	11.5	50.3
11	11	33	1	1	9.1	11	27	6	6	15.7	5.1	39.0	0.0	0.0	0.0	2.0	0.3	12.2
12	11	33	5	8	45.4	9	20	4	4	12.2	3.7	33.3	9.1	2.3	30.1	6.7	1.8	21.5
13	11	33	2	2	18.2	11	36	1	1	31.6	13.8	57.1	1.7	0.2	13.5	19.7	6.0	48.5
т	137	411	39	50	28.5	127	438	64	89	29.0			3.9			12.9		

Note: Communities: Canabrava = 1; Extrema = 2; Itajá II = 3; João de Deus = 4; Lageado = 5; Landi = 6; Pelotas = 7; Rochedo = 8; Fazenda Santo Antônio da Laguna = 9; São Lourenço = 10; São Sebastião da Garganta = 11; Sumidouro = 12; Taquarussu = 13; Household identification = ID; Upper limit = UP; Inferior limit = IL; residents quantity = Res; breeding sites quantity (Q) independent of the breeding site type = (a); quantity households with suspicious breeding sites (%) = (b); Breeding site favorable quantity by community = (c); Yes quantity = Y; dengue = DENV; Zika = ZIKV; chikungunya = CHIKV; seroprevalence - secondary data extracted from Pagotto et al (2022) = (d).

Regarding the possibility of finding breeding sites, their presence was observed at 92.7% (127/137) of visited households. However, 39 of them (28.5%), belong to 92.3% (12/13) of the studied communities, where 50 containers were found containing Culicidae living forms, characterized as suspicious *Aedes aegypti* in different stages (larvae, pupae and adult form). It is important to note that this situation was not found only at Landi community (6). Seroprevalence of 28.5% is similar to 29.4% found by Ruiz-Díaz et al (2017) in rural communities of the Caribbean coast.

Figure 5 shows breeding sites quantity with suspicious *Aedes aegypti* live forms (larvae, pupae and/or adult form) of households peridomicile of each community, comparing it to the number of potential breeding sites for culicid vectors reproduction, under the same environment. In 46.1% of the studied communities (6/13), BSSAaegypti was higher in relation to the PPBS, particularly two communities: Extrema (2) and Sumidouro (12) (Fig. 5).

Table 3 presents Pearson's correlation coefficients with statistical significance. Pearson coefficients showed positive relationship (p < 0,05) between BSSAaegypti and PPBS, at household (PPBSH) and community (PPBSC) levels. In an analysis of Table 3 in parallel with Table 2, Extrema (2) community was the one with the highest number of individuals affected by dengue (82.4%), with 85.7% (6/7) of the related variables significantly ($0.61 \le "r"$ "Pearson" ≤ 1.0), highlighting the relationships between SD_Z_C and SD, and with PPBSH, "r" "Pearson" = 1.0 and "r" "Pearson" = 0.67, respectively.

Among the 46.1% (6/13) PPBSC (Supplementary material and Fig. 5), 66.7% (4/6) of them showed relationship (p < 0.05) between PPCD, PPCC and/or BSSAaegypti) and SD_Z_C. On Table 3 can be highlighted that PPBSC x SD_Z_C [p < 0.05; 0.81] in Sumidouro; PPBSC x SD_Z_C [p < 0.05; 0.72] in Pelotas and BSSAaegypti x SD_Z_C [p < 0.05; -0.76] in Fazenda Santo Antônio da Laguna.

Table 3 Relationship between seroprevalence for dengue and/or Zika and/or chikungunya, and the number of breeding sites for culicid vectors, presented by Pearson's correlation coefficient.

RC	SD_Z_C		PPBSH		PPBSC				BSSAaegypti					
	SD	SC	SD_Z_C	SD	SD_Z_C	SD	SC	PPBSH	SD_Z_C	SD	SC	PPBSH	PPBSC	
	$\mathrm{r}_{\mathrm{Pearson}}$		$\mathbf{r}_{\mathrm{Pearson}}$		$\mathrm{r}_{\mathrm{Pearson}}$				$\mathrm{r}_{\mathrm{Pearson}}$					
1	-	0.77**	-	-	-	-	-	-	-	-	-	-	-	
2	1.00***	-	0.67**	0.67**	-	-	-	0.67**	-	-	-	0.61*	0.66**	
3	0.67**	1.00***	-	-	-	-	-	0.62**	-	-	-	-	0.57*	
4	-	1.00***	-	-	-	-	-	-	-	-	-	-	-	
5	0.85***	-	-	-	-0.55**	-0.66**	-	-	-	-	-	-	-	
6	1.00***	-	-	-	-	-	-	-	-	-	-	-	-	
7	1.00***	0.67**	-	-	0.72**	0.72**	0.69**	-	-	-	-	-	-	
8	0.57**	0.68***	-	-	-	0.44*	-	-	0.65***	-	0.48**	-	-	
9	1.00***	-	-	-	-	-	-	-	-0.76**	-0.76**	-	-	-	
10	0.56*	-	-	-	-	-	-	0.53*	-	-	-	-	-	
11	0.83***	-	-	-	-	0.52*	-	-	-	-	-	-	-	
12	-	0.62**	-	-	0.81***	-	0.59*	0.64**	-	-	-	-	0.53*	
13	1.00***	-	-	-	-	-	-	-	-	-	-	-	-	

Note: rural community = RC, names are shown in Table 2; breeding sites with suspicious forms (larvae, pupae and/or adult) of *Aedes aegypti* = BSSAaegypti; presence of possible breeding sites per household = PPBSH; presence of possible breeding sites per community = PPBSC; seroprevalence for dengue = SD; Zika seroprevalence = SZ; seroprevalence for chikungunya = SC; seroprevalence for dengue and/or Zika and/or chikungunya = SD_Z_C; non-significant correlation = (-); significance level: (***) = p-value ≤ 0.01 , (**) = p-value ≤ 0.05 , (*) = p-value ≤ 0.1 .

In the same context, Table 4 presents the distances (Km) from each community to the nearest urban center, with an average distance of 31.5 km, ranging from 6.7 to 82.8 km (Variation Coefficient = 66%; Standard deviation = 20.9 km). Comparing with the capital of Goiás state, Goiânia (where the research base was located), the average distance was 339.8 km, ranging from 94.6 to 626.2 km (Coefficient of variation (CV) = 51%; Standard deviation = 174.1 km). Household density per community is also shown, with averages, SD and CV of the distances between households, as well as the average and proportions of Culicidae breeding sites and seroprevalence, data used for Cluster analysis.

Besides the fact that those 13 rural communities studied here, demonstrated different typologies and geographical aspects in Goiás, Brazil; they share several common peridomiciles environmental features, capable of maintaining *Aedes aegypti* life cycle and, consequently, promote arboviral diseases dissemination.

Table 4

Distances between rural communities, the nearest urban center and Goiás state capital (Goiânia), the number of households per km² and of the variables

Rural	Distance (km)		Household/	Distance househole			Variable						
community	To	To state capital (Goiânia)	KIT-	Average (m)	STD (m)	CV (%)	PPBSC	PPBS	BSSAaegypti	SD	SZ	SC	SD_Z_C
	closest urban center						Average	Breeding sites			Soroprevalence		
	Center							proport	proportion		propo	proportion	
1 Canabrava	56.8	429.1	2.20	278.40	392.45	141	3.13	1.00	0.13	0.25	0.00	0.50	0.63
2 Extrema	6.7	515.1	43.62	43.00	40.52	94	3.20	0.80	0.60	0.90	0.10	0.10	0.90
3 Itajá II	9.5	192.4	4.03	278.20	121.57	44	1.82	0.73	0.18	0.09	0.00	0.18	0.18
4 João de Deus	25.2	108.1	2.67	297.10	99.70	34	2.29	1.00	0.14	0.00	0.00	0.14	0.14
5 Lageado	37.5	518.5	2.19	446.10	187.62	42	3.38	0.85	0.23	0.54	0.15	0.15	0.62
6 Landi	82.8	433.5	8.68	177.40	138.12	78	3.83	1.00	0.00	0.83	0.00	0.17	0.83
7 Pelotas	41.1	545.9	3.55	14.90	7.87	53	3.80	1.00	0.60	0.20	0.00	0.10	0.20
8 Rochedo	16.7	94.6	2.95	354.50	70.91	20	4.37	1.00	0.32	0.16	0.00	0.21	0.37
9 Fazenda Santo Antônio da Laguna	37.8	282.9	3.17	470.80	204.68	43	5.44	1.00	0.22	0.67	0.00	0.33	0.67
10 São Lourenço	42.4	279.7	3.10	346.20	134.63	39	2.27	0.91	0.36	0.45	0.27	0.27	0.73
11 São Sebastião da Garganta	28.8	139.8	2.10	296.60	158.59	53	2.45	1.00	0.09	0.45	0.00	0.09	0.55
12 Sumidouro	14.5	251.9	1.72	206.00	202.83	98	1.82	0.82	0.45	0.09	0.09	0.18	0.36
13 Taquarussu	10.1	626.2	6.29	247.20	151.77	61	3.27	1.00	0.18	0.09	0.00	0.00	0.09

Note: sites with suspected *Aedes aegypti* = BSSAaegypti; proportion of possible breeding sites in the community = PPBS; average of possible breeding sites per household in the community = PPBSC; seroprevalence for dengue = SD; seroprevalence for Zika = SZ; seroprevalence for chikungunya = SC; seroprevalence for dengue, Zika and/or chikungunya = SD_Z_C; standard deviation = STD; coefficient of variation = CV.

In Fig. 6a, a dendrogram from cluster analysis is presented, where communities are aggregated in four homogeneous clusters, according to the similarity between their characteristics (Fig. 6b). Canabrava (Cluster 1) and Extrema (Cluster 2) communities were isolated according grouping analysis. This might be possible due to their unique characteristics and differences. In Extrema, their population density is a higher (43.62 hh/km²), followed by the Landi (8.68 hh/km²). Extrema, also, showed greater proximity between households, as well as elevated arboviruses dissemination and breeding sites presence, corroborated by its Pearson correlation coefficient. In Canabrava, population density was low (2.2 hh/km²) and it was one of the communities with the lowest number of significant Pearson correlation coefficients.

The other two Clusters (3 and 4) where conformed by 5 and 6 communities, respectively. In relation to Cluster 4, a greater variation prevailed in all parameters, excepting population density (1.72 to 6.29 hh/km², CVg3 = 47.05%; CVg4 = 67, 16%) and the number of BSSAaegypti (0.18 to 0.60, CVg3 = 65.20%; CVg4 = 67.05%). Cluster 4 was confirmed by Rochedo, Fazenda Santo Antônio da Laguna, Lageado, São Lourenço, Landi and São Sebastião da Garganta, communities characterized by the substantial dispersion of households (dmean = 348.60 m, CV = 30.49%) and a notably low breeding sites CV (CV PPBS = 6.91%), including the lowest variation in seroprevalence for the three arboviruses, demonstrating that these parameters are crucial for studies on this topic.

Final considerations

Dengue is the arbovirus that most infected the population at some point in its life, compared to other arboviruses (Zika and chikungunya), and may be associated with the co-circulation of different serotypes of the virus (DENV-1-4) in the Brazilian territory, as well as *Aedes aegypti* transmissibility efficiency (Brazil 2009; Novaes, Pinto and Marques 2022). One of the ways to mitigate the transmission of these arboviruses is by combating *Aedes aegypti*, in which different insecticides have been tested against the vector, such as: ursolic acid (Kamatchi et al 2023), emodin (Chinnasamy et al 2023), isolated compounds (Raguvaran et al 2023), among others. However, bioefficacy against the vector can be compromised due to the socio-environmental variables that contribute to mosquito proliferation in different regions. In Brazil favored is vector, either by the adequate environment for its habitat and/or the lack of

basic sanitation services universalization (Morgan, Strode and Salcedo-Sora 2021; Novaes, Pinto and Marques 2022), as informed in the literature, mainly in Brazilian rural areas.

The studied data in the present research revealed that in 48.7% of the households (19/39), where infection by dengue, Zika and/or chikungunya was confirmed by seroprevalence, at least one water container with suspected *Aedes aegypti* was registered. Although one community (Landi) did not contain breeding sites with suspicious *Aedes* live forms, potential breeding sites were found in 5.3% of the households (23/438), in addition to the expressive seroprevalence rates for the three arboviruses, emphasizing the second highest value for Zika (23.4%) and the third highest value for dengue (63.7%).

In 30.8% of the communities, more than 1/3 of the households showed immatures and adults suspicious *Aedes aegypti* live forms, including São Lourenço community (10), where the highest seroprevalence for chikungunya was observed (26.6%).

Chikungunya was the only arbovirus that in all 13 communities at least one household had been infected. One of the reasons for this scenario may be due its spread in Brazil, prevailing two species of mosquitoes that transmit the disease: *Aedes aegypti* and *Aedes albopictus* (Honório et al. 2015; Souza et al. 2022; Almeida et al. 2022).

It was found in the peridomicile of 73 households, where everyone tested had no previous contact with at least one of the arboviruses mentioned (DENV, ZIKV and CHIKV), 26.0% (19/73), the presence of at least one container with immature and adult suspicious *Aedes aegypti* live forms. This was observed in 69.2% (9/13) of the communities, which could serve as warning to municipal health authorities. The relationship between the number of breeding sites found (PPBS, PPBSC and/or BSSAaegypti) with at least one of the three arboviruses, was observed in 53.8% (7/13) of the communities (Table 3). Therefore, it can be demonstrated that in a significant portion of the rural communities visited (53.8%), the breeding sites found in peridomicile location were probably Culicidae vector habitat, responsible for infecting part of their population regardless of, whether or not, mosquito identification during this investigation.

Thus, confirming the presence of *Aedes aegypti* or *Aedes albopictus*, vectors carrying an arbovirus, after biting an infected person coming mainly from the urban area, at least 25% of the families in at least 69.2% (9/13) of the rural communities studied, can be affected due the possible vector proliferation in rural areas on Brazilian Midwest.

Therefore, better evaluation is recommended, especially regarding entomological focus in order to increase vectors understanding and their dispersion in this region.

This research is limited by the seroprevalence data obtained from late infections, it means that the studied population had previously contact with, at least, one of the arboviruses, at some point in their lives, as a methodology commonly used in other studies (Brunkard et al. 2007; Siregar et al. 2015; Elaagip et al. 2020; Tellle et al. 2021). However, the communities that participated in the present research are far from urban centers (in average 31.5 km), which can lead to similar results in quantity of breeding sites presence, containing Culicidae individuals and infected people at that moment.

Conclusions

This work allowed to conclude that:

• The knowledge of residents in peridomestic conditions on rural communities, affected by arboviral diseases, showed better understanding of the real possibility of vectors, to establish themselves in rural areas of the Brazilian Midwest. This is mainly due to several environments promoting culicids proliferation, with medical concerns on this region;

• Arboviruses dissemination may have occurred within the community itself, considering that arboviral diseases dengue, Zika, and chikungunya affected a significant portion of its population. The high seroprevalence rates are highly correlated, statistically, with the breeding sites quantity found in the peridomiciles.

• This research opens up the possibility to infer that certain rural communities in the state of Goiás may maintain the manifestation of high seroprevalence rates for these arboviruses, which might constitute a risk to future generations in these regions;

• A community lacking basic sanitation facilities, among other factors, is more susceptible to the presence of disease vectors, since breeding sites found (in general water tanks, buckets, plastic drums, solid waste, tires) mainly from the precarious or intermittent water supply, and improper solid waste handling. Thus, universalization of public sanitation policies is essential for disease prevention.

Therefore, the present study, in addition to instigate new reflections on this subject, may serve as a guide for vector control actions in rural areas.

Declarations

Supplementary Materials The following supporting information can be downloaded at: (link) Supplementary material: Breeding sites quantity with and without suspicious of *Aedes aegypti* and seroprevalence for dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV), in rural communities in Goiás, Brazil.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Figures

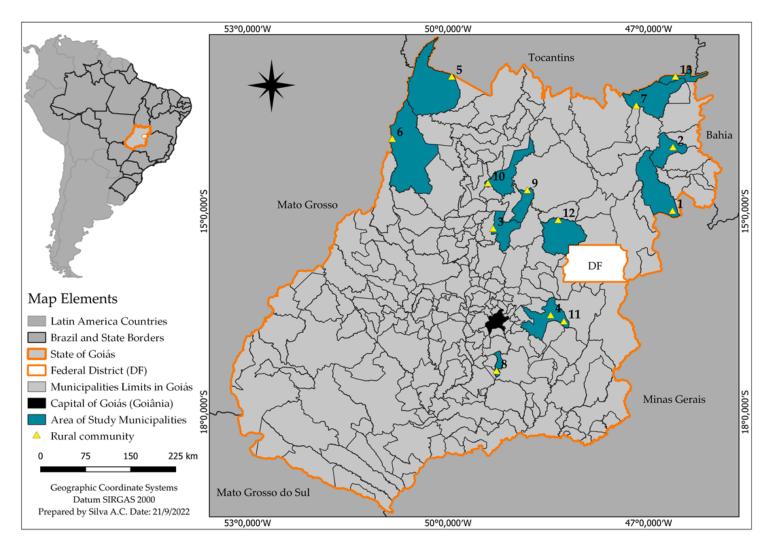


Figure 1

Rural communities' location and their respective municipalities in Goiás, Brazil. Its capital Goiânia is highlighted in black, where the laboratory analysis was developed. Source: Created by authors. Note: Community/Municipality: Canabrava/Flores de Goiás = 1; Extrema/laciara = 2; Itajá II/Goianésia = 3; João de Deus/Silvânia = 4; Lageado/São Miguel do Araguaia = 5; Landi/Santa Rita do Novo Destino = 6; Pelotas/Monte Alegre de Goiás = 7; Rochedo/Professor Jamil = 8; Fazenda Santo Antônio da Laguna/Barro Alto = 9; São Lourenço/Uruaçu = 10; São Sebastião da Gargan-ta/Silvânia = 11; Sumidouro/Padre Bernardo = 12; Taquarussu/Campos Belos = 13.

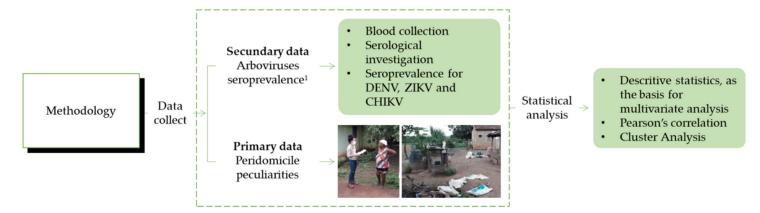


Figure 2

Research development flowchart. Note: 1 = Data from Pagotto et al (2022).



Figure 3

Visits to rural communities between October/2021 and December/2021 with application a survey (a) and registration of potential mosquito breeding sites (b-f).

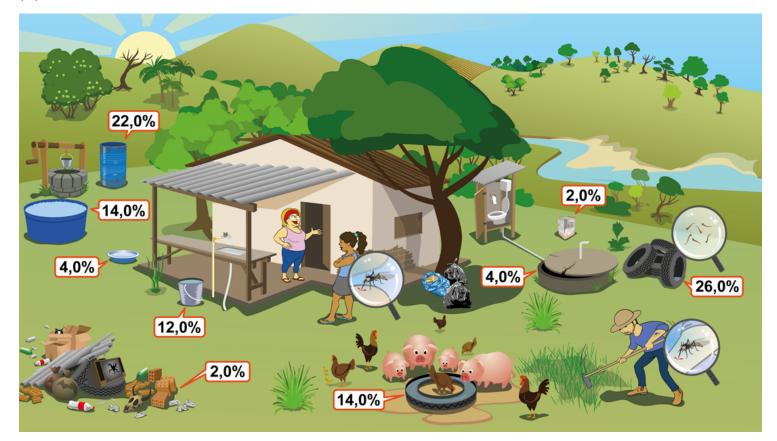


Figure 4

Schematic drawing, identifying the breeding sites found and their respective percentages of occurrence.

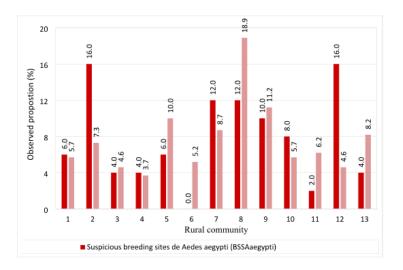


Figure 5

Sites with suspicious Aedes aegypti live forms, compared to possible sites for culicid vectors proliferation in the peridomicile of rural communities in Goiás, Brazil. Note: Canabrava = 1; Extrema = 2; Itajá II = 3; João de Deus = 4; Lageado = 5; Landi = 6; Pelotas = 7; Rochedo = 8; Fazenda Santo Antônio da Laguna = 9; São Lourenço = 10; São Sebastião da Garganta = 11; Sumidouro = 12; Taquarussu = 13.

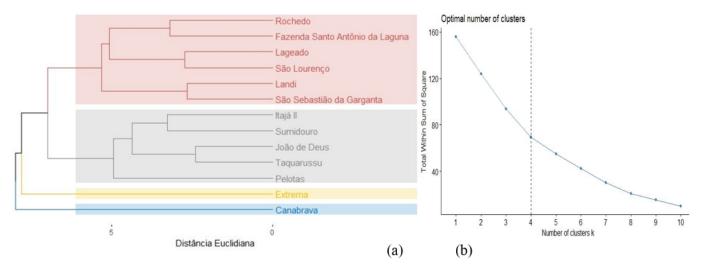


Figure 6

Sites with suspicious *Aedes aegypti* live forms, compared to possible sites for culicid vectors proliferation in the peridomicile of rural communities in Goiás, Brazil.

Supplementary Files

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