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ORIGINAL ARTICLE

ENTOMOVIROLOGICAL INVESTIGATION OF MOSQUITOES (DIPTERA: CULICIDAE) IN AREAS WITH YELLOW FEVER EPIZOOTICS IN GOIÂNIA, GOIAS, BRAZIL

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ABSTRACT

The objective of this investigation was to analyze the occurrence and the natural infection of mosquitoes in areas with epizootics of non-human primates (NHP) confirmed for yellow fever in the city of Goiânia, Goiás, Brazil from November 2020 to April 2021. This is an ecological, descriptive, retrospective study, with quantitative and qualitative data on the mosquito fauna collected in 10 areas. A total of 2,787 Culicidae were collected and allocated in eight genera and 23 species. Of the total specimens captured, 2,341 (84%) were females and 446 males (16%). *Haemagogus janthinomys* occurred in 2.7% (74) of the cases and *Hg. leucocelaenus* in 4.5% (125); *Sabethes chloropterus* occurred in 0.1% (3) of the cases, *Sa. albiprivus* in 1% (28) and *Sa. glaucodaemon* in 0.3% (8); *Aedes aegypti* occurred in 19.7% (548) and *Ae. albopictus* in 3.9% (108) of the cases. The *Sa. glaucodaemon* species was recorded for the first time in Goiânia. Yellow fever infection was not detected in the collected specimens. We concluded that the presence of wild vectors, in addition to potential urban vectors, enabled the classification of receptive and vulnerable areas to the circulation of the yellow fever virus.

KEY WORDS: Entomological surveillance; vector mosquitoes; epizootics; yellow fever; arbovirology; hemorrhagic fevers

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INTRODUCTION

Yellow Fever (YF) is an acute febrile infectious disease whose etiological agent is an arbovirus which consists of single-stranded RNA of the genus *Flavivirus*, and it is transmitted through the bite of infected female mosquitoes (Walker et al., 2020).

Around 200,000 cases of YF are detected per year worldwide, with an estimated of 30,000 deaths. The cases currently occur in 34 countries in Africa, 11 in South America and two in Central America (PAHO, 2021). The urban YF cycle was eliminated in 1942 in Brazil (Galindo et al., 1956; Costa et al., 2011) after the introduction of the vaccine in 1937 and the intensification of the efforts to combat the urban vector – *Aedes aegypti*, which, after a blood meal, may become infected and transmit the disease to humans (Souza-Neto et al., 2019). From then on, only the sylvatic cycle occurs, with transmission of the YF virus (YFV) to humans by mosquitoes of the genus *Haemagogus* and *Sabethes*, preceded by the occurrence of epizootics of non-human primates (NHP) (Silva et al., 2020).

From July 2014 to June 2019, the Ministry of Health confirmed the occurrence of 2,260 human cases of YF, with 773 deaths and a fatality rate of 34.2%, in addition to 2,670 epizootics of non-human primates (NHPs) in Brazil (Ministério da Saúde, 2019). Ten human cases and 27 epizootics occurred in the State of Goiás, with 1 (10%) human case and 11 (40%) epizootics in Goiânia – the capital of the State of Goiás (SES-GO, 2021). The city of Goiânia has an estimated population of 1,555,626 inhabitants, a total area of 728,841 km² and a demographic density of 1,776.74 inhabitants/ km². The relief is composed of altitudes ranging from 550 to 1,100 m. The vegetation in Goiânia comprises forest, grassland and savannah formations belonging to the Cerrado biome (Goiânia, 2019; IBGE, 2021).

The new YF cycle in the extra-Amazonian region began with the detection of confirmed epizootics in the State of Goiás and in the Federal District during the monitoring period from July 2020 to June 2021 (Ministério da Saúde, 2021a). In this new surveillance cycle, 21 epizootics were confirmed, without any confirmation of human cases in Goiás so far (SES-GO, 2021).

Early detection of viral circulation through epizootic surveillance of NHP and entomological culicids allows for the delimitation on the areas at risk of transmission of the YFV, and the targeting of immunization to populations at greater risk (Ministério da Saúde, 2017; Abreu et al., 2019a; Ministério da Saúde, 2021b). The current epidemiological situation demands greater sensitivity of entomological surveillance, aiming at timely identifying the re-urbanization risk (Figueiredo et al., 2020). The sylvatic YF transmission cycle involves the natural infection of vectors of the *Haemagogus* and *Sabethes* genera that become infected when they have a blood meal in NHPs during the viremia period of the disease (Shannon et al., 1938; Rodaniche & Galindo, 1957). These vectors occasionally feed on humans, who may become ill (Pinto et al., 2009; Costa et al., 2011).

The main species of mosquitoes with detected natural infection and vectorial potential for YF transmission in the Americas are *Hg. (Hg.) janthinomys, Hg. (Conopostegus) leucocelaenus,* and *Sa. (Sa.) chloropterus* (Boshell-Manrique & Osorno-Mesa, 1944; Goenaga et al., 2012; Abreu et al., 2019b). *Ae. aegypti* has not been found infected with the YFV in recent years, but the risk of YF re-urbanization exists due to the large number of unvaccinated people who visit areas with circulation of the YFV (Pinheiro et al., 2019).

Considering the importance of entomological investigation in environments with reports of NHP epizootics linked to cases of YF, the survey and the monitoring of Culicidae is one of the pillars of the YF surveillance program, which may allow studies of the ecoepidemiology of this disease. In the State of Goiás, there is a need for execution and for publishing official records that describe the main species raised and the viral detection in mosquitoes in areas of epidemiological relevance for the YFV transmission. The data presented in this study will make it possible to improve YF surveillance, the prevention measures in the State and the direct vector control actions toward potential YFV vector species.

The aim of this study was to analyze the natural infection and the occurrence of mosquito species collected in areas with confirmed NHP epizootics in Goiânia, Goiás, during the monitoring period from November 2020 to April 2021.

MATERIAL AND METHODS

The areas selected for collection were classified as Permanent Protection Areas (APPs), in hydrographic areas with high erosive potential due to slope and in Conservation Units with primary, riparian and degraded forest.

Culicidae collections were conducted from 11/18/2020 to 4/27/2021, from 9:00 am to 3:00 pm, in 10 areas located in 10 different neighborhoods, which had ecological and environmental conditions favorable to the presence of the mosquito. Mosquito collections were distributed according to the type of vegetation, as follows: i) eight collections in three primary forest areas; ii) 22 collections in six degraded forest areas, and iii) one in riparian forest area.

The collections were done by technicians from the Zoonosis Surveillance Board using the protected and clarified human attraction technique (ATHPE) on the ground, an entomological net, and a Castro trap placed in an entomological pot. Between 1/12/2020 and 10/12/2020, the collections were done using 06 BG-sentinel traps installed on the ground, as well as in the tree canopy.

Adult mosquitoes captured with BG-Sentinels and actively captured (Castro Trap and entomological net) were immediately killed on dry ice and kept at -80 ºC until they were tested for YFV. The identification of mosquitoes was carried out by directly observing morphological characters under a stereomicroscope (Zeiss®) on a cold table, following the methods reported by Arnell (1973), Consoli & Lourenço-de-Oliveira (1994), Forattini (2002), Marcondes & Alencar (2010) and Abreu et al. (2019b). The morphological identification of mosquitoes was performed on the same day of collection.

Subsequently, they were sent to the Central Public Health Laboratory of Goiás (LACEN-GO) to be sent to the network's reference laboratories. Molecular diagnosis and confirmation of species that were potentially vectors of YFV were done by the Hematozoan-transmitting Mosquito Laboratory (LATHEMA) from the Instituto Oswaldo Cruz (IOC/FIOCRUZ) and the Molecular Biology Laboratory from the Section of Arbovirology and Hemorrhagic Fevers at the Evandro Chagas Institute (IEC).

The analytical procedure to detect the presence of YFV was almost exclusively performed on non-blood-fed female mosquitoes (tribes Aedini and Sabethini) that were caught actively and with BG-Sentinel. Briefly, mosquitoes belonging to the same species, same sampling site and same date were pooled (≤10 individuals each), homogenized in 1,000µL of L-15 culture medium supplemented with 4% fetal bovine serum, and centrifuged at 10,000g for 10 min at 4 °C. RNA was extracted from the supernatant by using the QIAamp Viral RNA Mini Kit, according to the manufacturer's instructions. The extracted RNAs were tested in duplicate by RT-qPCR using the set primers and protocols described previously (Domingo et al., 2012; Abreu et al., 2019b; de Miranda et al., 2022).

The UTM Sirgas 2000 zone 22S coordinates were used for georeferencing culicid collections and YF epizootics confirmed in the urban and rural landscape from the city of Goiânia. This landscape was mapped by supervised classification of high-resolution satellite images CBERS-4A (INPE, 2022). The supervised classification protocol used here was previously described in another study (Ilacqua et al., 2018) and recently applied to the re-emergence of YF in Brazil (Ilacqua et al., 2021).

RESULTS

A total of 2,787 specimens were collected and distributed in eight genera and 26 species of Culicidae, as described in Table. Of the total specimens captured, 2,341 (84%) were females, and 446 were males (16%), with higher frequencies for *Psorophora albigenu* and *Ae. aegypti*. The highest number of mosquitoes was collected in the Jardins Paris area, corresponding to 61%. Collections carried out in the area located in Vila Osvaldo Rosa contributed to 17% of the total number of mosquitoes. The other areas of the study together represented 22% of the overall abundance (Figure 1). The mean interval between mosquito collections and reporting of epizootics was 37 days.

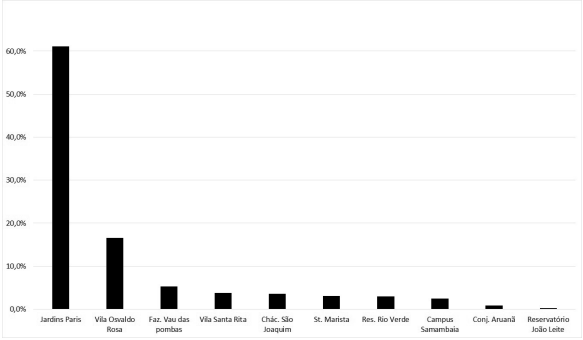

Figure 1. Distribution of relative abundance (%) of mosquitoes collected at ten selected sites in the city of Goiânia, GO, 2020-2021.

The Figure 2 shows that *Ps. albigenu* had the highest occurrence of 43.4% (1,212). However, regarding YF vectors, *Hg. janthinomys* and *Hg. leucocelaenus*, potential primary vectors, were collected mainly in areas of primary forest, with an abundance of 2.7% (74) and 4.5% (125), respectively (Table). Potential secondary vectors, *Sa. glaucodaemon* with 0.3% (8) and *Sa. chloropterus*, with 0.1% (3) abundance, occurred in greater proportions in degraded forest areas (Table). *Sa. albiprivus* with 1% (28) was collected in a similar proportion in areas of primary or degraded forest (Table). *Hg. janthinomys* (2.7%) and *Hg. leucocelaenus* (4.5%) occurred in greater proportions in primary forests, with high coverage of trees, shrubs, and grasses. *Sa. glaucodaemon* (0.3%) and *Sa. chloropterus* (0.1%) were collected in degraded forests with the presence of nearby housing, low shrub coverage and few trees. On the other hand, *Sa. albiprivus* (1%) was collected in a similar proportion in areas of primary or degraded forest.

Ae. albopictus, with a frequency of 3.9% (108), was collected mainly in degraded forest areas, while *Ae. aegypti* with 19.7% (548) was also detected in large numbers in primary forest areas (Table), mainly on the edges, which are close to the houses.

The RT-PCR test was performed on pools of mosquitoes belonging to the genera *Aedes, Haemagogus, Sabethes* and *Psorophora*. All the results of the processed samples were negative for the presence of the YFV genome. Viral detection was performed by the IEC laboratory, which processed 155 specimens, and LATHEMA (IOC/FIOCRUZ), which processed 2,275 specimens.

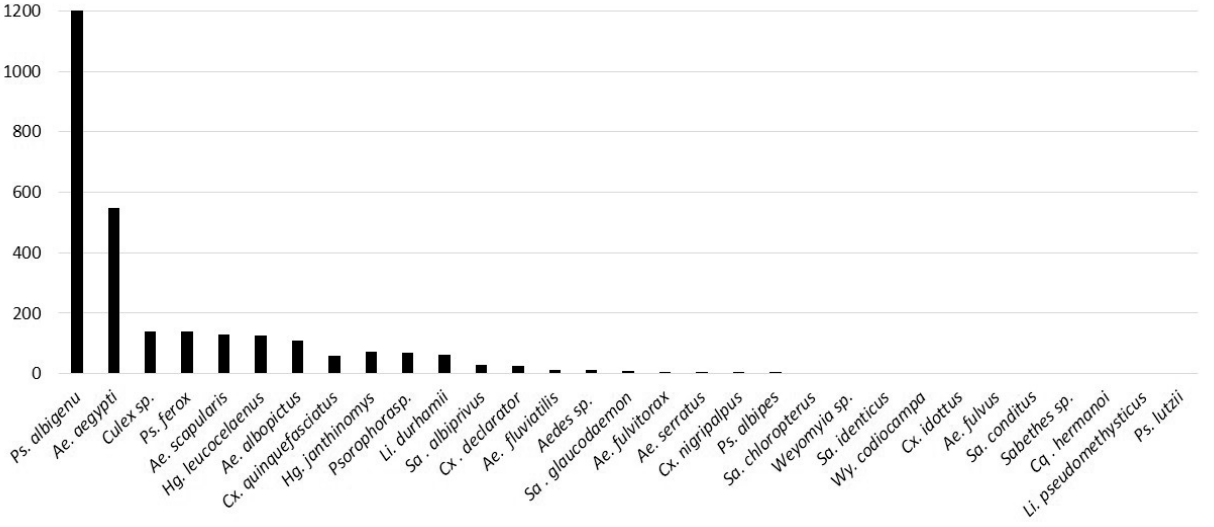

Figure 2. Occurrence of Culicidae species in the city of Goiânia, GO, during the entomological investigation of YF vectors, 2020-2021.

Table. Distribution of the species collected at 10 collection sites in the city of Goiânia/GO according to the type of vegetation during the YF virus circulation period, 2020-2021.

Species	n	Frequency (%)	Type of vegetation		
			Primary forest	Riparian forest	Degraded forest
Ps. albigenu	1,211	43.5	1,062 (87.7%)	52 (4.3%)	97 (8.0%)
Ae. aegypti	548	19.7	299 (54.6%)	–	249 (45.4%)
Culex sp.	138	5.0	14 (10.1%)	–	124 (89.9%)
Ps. ferox	140	5.0	55 (39.3%)	47 (33.6%)	38 (27.1%)
Ae. scapularis	130	4.7	104 (80%)	1 (0.8%)	25 (19.2%)
Hg. leucocelaenus	125	4.5	116 (92.8%)	–	9 (7.2%)
Ae. albopictus	108	3.9	19 (17.6%)	1 (0.9)	88 (81.5%)
Cx. quinquefasciatus	60	2.2	1 (1.7%)	–	59 (98.3%)
Hg. janthinomys	74	2.7	64 (86.5%)	2 (2.7%)	8 (10.8%)
Psorophora sp.	69	2.5	2 (2.9%)	39 (56.5%)	28 (40.6%)
Li. durhamii	62	2.2	11 (17.7%)	–	51 (82.3%)
Sa. albiprivus	28	1.0	16 (57.1%)	–	12 (42.9%)
Cx. declarator	27	1.0	–	–	27 (100%)
Ae. fluviatilis	12	0.4	3 (25%)	–	9 (75%)
Aedes sp.	11	0.4	7 (63.6%)	–	4 (36.4%)
Sa. glaucodaemon	8	0.3	–	–	8 (100%)
Ae. fulvitorax	6	0.2	6 (100%)	–	–
Ae. serratus	7	0.3	–	5 (71.4%)	2 (28.6%)
Cx. nigripalpus	4	0.1	–	–	4 (100%)
Ps. albipes	4	0.1	2 (50.0%)	–	2 (50.0%)
Sa. chloropterus	3	0.1	1 (33.3%)	–	2 (66.7%)
Weyomyia sp.	2	0.1	–	–	2 (100%)
Sa. identicus	2	0.1	–	–	2 (100%)
Wy. codiocampa	1	0.0	–	–	1 (100%)
Cx. idottus	1	0.0	–	–	1 (100%)
Ae. fulvus	1	0.0	–	–	1 (100%)
Sa. conditus	1	0.0	–	–	1 (100%)
Sabethes sp.	1	0.0	–	–	1 (100%)
Cq. hermanoi	1	0.0	–	–	1 (100%)
Li. pseudomethysticus	1	0.0	–	–	1 (100%)
Ps. lutzii	1	0.0	–	–	1 (100%)
Total	2,787	100.0	1,783 (64.0%)	147 (5.3%)	857 (30.7%)

Mapping showed that most mosquito collections were carried out in areas where epizootics of NHPs were confirmed for YF (Figure 3). The distribution of potential YF vector species collected according to their occurrence in the ten collection points was in the following proportion: *Hg. janthinomys* in 90% and *Hg. leucocelaenus* in 80% of the locations. When added together, they occurred in 100% of the surveyed areas: *Sa. chloropterus* in 40%, *Sa. glaucodaemon* in 20% and *Sa. albiprivus* in 60%, with the occurrence of at least one of these species in 80% of the locations. *Ae. albopictus* was detected in 90% and *Ae. aegypti* in 70% of the collection points (Figure 4).

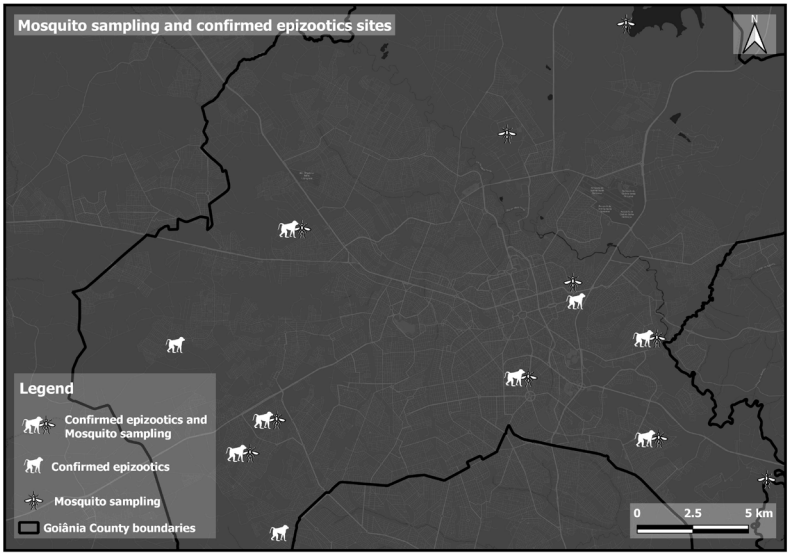

Figure 3. Distribution of confirmed epizootics in PNH (*Alouatta caraya, Callithrix penicillata,* or *Sapajus libidinosus*) and collection sites of potential yellow fever virus vectors (*Haemagogus, Sabethes,* and *Aedes*) in the city of Goiânia, GO, 2020-2021.

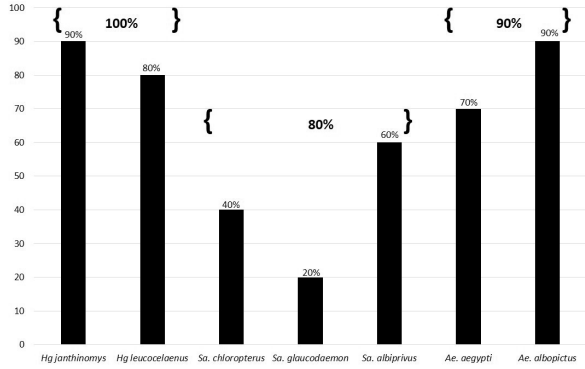

Figure 4. Distribution of potential YF vector species according to occurrence in the ten collections sites, city of Goiânia/ GO, 2020-2021. The values in bold represent the sum of the species of the genera.

DISCUSSION

In the Central-West region, the species *Hg. janthinomys, Hg. leucocelaenus, Sa. chloropterus* and *Sa. albiprivus* have already been detected in areas of the Federal District (Obara et al., 2012; Lira-Vieira et al., 2013). These results also agree with the records of this study, except for *Sa. glaucodaemon* and *Sa. conditus*, whose occurrences in the city of Goiânia, Goiás, had never been registered before. The frequencies found in this study can be compared to other studies that detected the YFV in mosquitoes from areas with confirmed NHP epizootics.

In the period from 2016 to 2018, the relative abundance of mosquito species collected before and during YFV circulation in cities in the Southeast Region of Brazil was 9% for *Hg. leucocelaenus*, 4% for *Hg. janthinomys*, 0.3% for *Sa. chloropterus*, 3% for *Sa. albiprivus*, 5% for *Ae. albopictus* and 0.7% for *Ae. aegypti* (Abreu et al., 2019b). These results were higher than the abundance found in this study, except for *Ae. aegypti*, which was 19.7%, probably because it has a high level of adaptation to modified environments that occur on the edge of forests in anthropized areas (Hendy et al., 2020), as in the case of some areas selected for this work.

In the Serra do Rola-Moça State Park (PESRM) and in the Fechos Ecological Station (EEF), located in the State of Minas Gerais, in 2017-2018, during the period of viral circulation, *Hg. janthinomys* presented a lower abundance than in this study, and YFV infection was not detected (Pinheiro et al., 2019). However, the abundance of 4.8% for *Hg. leucocelaenus*, 1.8% for *Ae. albopictus* and 0.5% for *Sa. albiprivus* corroborates the results in this present study (Pinheiro et al., 2019).

Hg. leucocelaenus (125) and *Hg. janthinomys* (74) occurred in greater numbers than mosquitoes of the genus *Sabethes*. This fact could be explained by the behavior of descending to the ground level, evidenced mainly in *Hg. leucocelaenus* (Deus et al., 2022). Another possible explanation would be the collection method used, as the use of a lure and Castro catcher presents a greater yield to capture culicids of the genus *Haemagogus* when compared to the BG-sentinel trap, however there is synergism when both techniques are performed (Deus et al., 2022).

The greater abundance of *Hg. leucocelaenus* and *Hg. janthinomys* in areas of primary forest corroborated the records of the last three decades, where their occurrence becomes greater in urban green areas and modified forest fragments, including small forests close to cultivated areas and pastures, usually associated with small rivers and streams (Li et al., 2022). For species of the genus *Sabethes*, the results of this study are close to those ones found in YF outbreak investigations between 2016 and 2018, which point to a certain limitation in ecological conditions with low distribution, abundance and infection rates (Li et al., 2022).

During periods with a viral circulation of the YFV in Altamira in the State of Pará, North Region of Brazil, *Hg. janthinomys* presented an infection rate of approximately 3.6% and 37.8% of the positive pools. The infection was also detected in nulliparous females, which confirmed the transovarian transmission of the virus in this species (Mondet et al., 2002). *Hg. leucocelaenus*, on the other hand, was incriminated as a primary vector in the cities of Caibaté and Coronel Barros, in the State of Rio Grande do Sul, after confirming natural infection in these species, in areas where *Hg. janthinomys* was absent (Cardoso et al., 2010). In the Central-West region, simultaneous infection has already been detected in the two species mentioned above, characterizing them as potential vectors (Obara et al., 2012). Natural infection in Culicidae species captured in 43 municipalities located in the States of São Paulo, Rio de Janeiro, Minas Gerais, Espírito Santo and one city in the State of Bahia, between 2016-2018, characterized *Hg. janthinomys* and *Hg. leucocelaenus* as primary vectors of YFV and *Sa. chloropterus* as secondary vector (Abreu et al., 2019b). Viral detection in mosquitoes was possible between three and 24 days after detecting the first traces of viral circulation (Abreu et al., 2019b). In this study, only four out of the 31 collections were performed in that interval. This fact may have contributed to the non-detection of the YFV in the samples analyzed.

The entomological investigation in the cities of São José do Rio Preto and Ribeirão Preto, in the State of São Paulo, registered the occurrence of the species: *Hg. janthinomys, Hg. leucocelaenus* and *Sa. chloropterus*. However, natural infection was detected in none of these species (Moreno et al., 2011), as reported in this research.

Collections and identifications carried out in the cities from the Southeast Region of Brazil detected mosquitoes of the species *Hg. leucocelaenus* in 71% of the areas and *Hg. janthinomys* in 57%, *Sa. chloropterus* in 29% and *Sa. albiprivus* in 43%, *Ae. albopictus* in 95% and *Ae. aegypti* in 29% (Abreu et al., 2019b). In this study, *Hg. janthinomys* and *Hg. leucocelaenus* were recorded in most of the surveyed areas, with the distribution of at least one of these species in all areas. Other species involved in YF transmission showed lesser distribution, such as *Sa. chloropterus* and *Sa. albiprivus*. Potential urban vectors, *Ae. albopictus* and/ or *Ae. aegypti* were detected in most locations.

Studies carried out at the PESRM and EEF located in the State of Minas Gerais and urban, peri-urban, rural, and wild areas in the Federal District did not identify the presence of *Ae. aegypti* (Obara et al., 2012; Pinheiro et al., 2019). In this research, this species was also not found in areas with a higher degree of preservation, such as in the forests of the Reservoir of Ribeirão João Leite, Fazenda Vau das Pombas and Campus Samambaia at Universidade Federal de Goiás.

Mapping enabled a synergistic association between the spread of the yellow fever virus, detected through positive epizootics, and the ecological corridors, characterized by the presence of riparian forest fragments in anthropized areas (Prist, 2021; Wilk-da-Silva, 2022). There was an overlapping geographic distribution/ dispersion of species' potential vectors of YF, positive epizootics and ecological corridors. The classification of areas enables a prompt response from entomological surveillance regarding the precise selection of collection points within the cities, especially when there are signs of viral circulation.

Our results demonstrate that the wide distribution of potential primary vectors in most locations, and the recurrent occurrence of positive epizootics point to the vulnerability and receptivity of the city of Goiânia for YF. These results increase the likelihood of new outbreaks of this disease. During the monitoring period, 21 epizootics were confirmed, without confirmation of human cases in Goiás. In Goiânia, 11 NHP epizootics were confirmed: five *Alouatta caraya*, four *Callithrix penicillata*, and two Sapajus libidinosus (SES-GO, 2021).

The main limitation of this study was the long interval between notification of epizootics and mosquito collections. Perhaps this fact has contributed to the non-detection of the virus in mosquitoes during the seasonal period of YF in the city of Goiânia. Mosquito collections must be carried out in a timely manner, and the detection of yellow fever infection in culicids be implemented in the routine of regional laboratories, with results in a timely manner, to help in decision-making regarding surveillance measures, prevention and control of arboviruses. The city of Goiânia has receptive and vulnerable areas with the possibility of new cases of yellow fever. Therefore, systematic and periodic monitoring of new areas is necessary to reduce the risk of YF transmission in Goiânia, Goiás, Brazil.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest or financial ties to disclose.

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