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REVIEW

NATURAL ARBOVIRUSES INFECTION IN IMMATURES AND ADULTS OF *Aedes* GENUS (DIPTERA: CULICIDAE) IN THE STATE OF MINAS GERAIS: A SYSTEMATIC REVIEW

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ABSTRACT

Arboviruses are considered a burden for public health systems around the world. In Brazil, a country that is hyperendemic for arboviruses, the State of Minas Gerais has recorded the second-highest number of probable dengue cases in 2024. This systematic review of literature on natural arboviruses infection in both immatures and adults of *Aedes* genus in Minas Gerais, Southeast Region of Brazil, adhered to the established standards of the Preferred Reporting Items in Systematic Reviews and Metanalyses (PRISMA). The studies were searched in the *Biblioteca Virtual em Saúde* (BVS), SciELO, and PubMed databases. Nine of 524 studies published between 1993 and 2024 were included. Both immatures and adults of *Aedes aegypti* accounted for the largest number of specimens captured (49.7%). The positivity rate of *Ae. aegypti* infected by Dengue virus (DENV) ranged from 0.18% to 32.1% and 5.5% to 16.9% for larvae and adults, respectively. Adult specimens of *Ae. aegypti* also tested positive for Chikungunya virus (CHIKV). Some specimens of *Ae. albopictus*, *Ae. scapularis*, *Ae. argyrothorax* and *Ae. serratus* tested positive for Yellow Fever virus (YFV). Among the specimens of *Aedes* collected in the State of Minas Gerais, *Ae. aegypti* presented the highest percentage of natural arbovirus infection. The majority of specimens analyzed were naturally infected with DENV.

KEY WORDS: Dengue virus; Chikungunya virus; Zika virus; Yellow Fever virus.

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INTRODUCTION

Arboviruses are considered a burden on public health systems worldwide due to their rapid geographic expansion. Despite having vertical and transfusion transmission routes, arthropods' greatest relevance lies in vector transmission (Dalpadado et al., 2022; Silva et al., 2022). In the Americas, *Aedes* (Meigen, 1818) genus stands out for its wide distribution and vector capacity for different arboviruses. In Brazil, the subgenera *Ochlerotatus*, *Stegomyia*, *Howardina* and *Protomacleaya* have been registered. The species of epidemiological importance are grouped into the subgenera *Ochlerotatus* and *Stegomyia*, the latter represented especially by the genus *Aedes*, being *Aedes aegypti* (Linnaeus, 1762) the target of intense national campaigns of vector control (Duvall, 2019; Bezerra et al., 2021; Silva et al., 2022).

Among the arboviruses naturally transmitted by female adults of *Aedes* genus are the four serotypes that cause dengue fever (DENV-1, DENV-2, DENV-3, and DENV-4 - *Flavivirus*), the chikungunya fever virus (CHIKV - *Alphavirus*), and the Zika virus (ZIKV - *Flavivirus*). More sporadically, the yellow fever virus (YFV - *Flavivirus*) is also transmitted by *Aedes* females in urban areas. The differential diagnosis among the diseases is a challenge for surveillance, as they occur simultaneously and present similar non-specific symptoms, such as fever, headache, red spots on the body, and joint pain (Duvall, 2019; Godoy et al., 2021; Skalinski et al., 2022).

Diseases transmitted by adults of *Aedes* genus have become a significant public health problem; dengue fever has presented successive epidemics in Central and South American countries since the end of the 1970s. Additionally, Chikungunya fever emerged in the Caribbean in 2013, and the international and national emergence of the ZIKV associated with microcephaly in Brazil in 2015 (Skalinski et al., 2022). Between the 1st and 26th Epidemiological Weeks (EW) of 2024, 10,954,194 cases of arboviruses were reported in the Americas, of which 10,576,561 (96.6%) were cases of dengue fever, 352,966 (3.2%) were cases of chikungunya fever and 24,667 (0.2%) were cases of Zika (PAHO, 2024a; PAHO, 2024b; PAHO, 2024c). Due to the historic dengue epidemic across the country, the municipal government of Belo Horizonte, the capital of Minas Gerais, declared a public health emergency. This measure is taken in response to the critical epidemiological scenario of arboviruses, including dengue, chikungunya, and Zika (Belo Horizonte, 2024).

The first records of autochthonous transmission in the State of Minas Gerais date back to 1987, in Pirapetinga (Sefuro et al., 1993), with the first epidemic recorded in 1996 in the Metropolitan Region of Belo Horizonte (Corrêa et al., 2005). Until the EW 26 of 2024, the State of Minas Gerais recorded 1,655,644 probable cases of dengue, of which 970,216 were confirmed, with 732 deaths also confirmed. This represents the second-highest number of arbovirus cases in the Southeast region and throughout the country, behind

only São Paulo (DATASUS, 2024; SES-MG, 2024). The first autochthonous case of chikungunya fever occurred in 2016, with 16,013 suspected cases registered in 2017, positioning the State in first place in national notifications (Bagno et al., 2019). The first case of Zika in the State was officially confirmed in 2016, with previous silent circulation of the virus since December 2015 (Iani et al., 2021). Until the EW 25 of 2024, the State already has 279 probable cases of Zika (DATASUS, 2024)

The complicated epidemiological scenario found both nationally and in the State of Minas Gerais is sustained by the simultaneous circulation of arboviruses and the presence of immature and adult vectors throughout most of the territory, establishing a major challenge for surveillance and control. Hence, it is vital to understand the trends involving the vector to implement active surveillance and control actions, such as strategic monitoring of vector populations, capture, and virology analyses. Therefore, the present study aimed to conduct a systematic literature review to analyze the available information regarding natural infection in immature and adult specimens of *Aedes* genus in Minas Gerais.

METHODS

Study area

Minas Gerais is one of the 27 federative units in Brazil, located in the Southeast Region of the country. It borders six States: São Paulo (to the south), Espírito Santo and Rio de Janeiro (to the east), Bahia (to the north), Goiás (to the west), and Mato Grosso do Sul (to the southwest). With a territorial area of 586,513,983 km^2 and 853 municipalities, it is the largest State in the Southeast Region and the fourth-largest in Brazil. With a population of 20,538,718 inhabitants and a density of 35.02 inhabitants/km^2, it is the second most populous State in the country (IBGE, 2023a). The most populated city in the State is the capital, Belo Horizonte, with 2,315,560 inhabitants (IBGE, 2023b).

The tropical climate predominates in the north and part of the State's northwest. This climate has regional subdivisions due to altitude, varying among high-altitude tropical and humid tropical. In these locations, temperatures are high throughout the year, with averages ranging between 22 °C and 28 °C, with hot and rainy summers. On the other hand, winters are mild, with less precipitation. Furthermore, the State has a semi-arid climate in part of the north, with high temperatures and annual averages above 28 ºC (Sagicapri, 2023). As for biomes, Minas Gerais has three main ones found in Brazil: Cerrado, Caatinga, and Mata Atlântica (Sagarana, 2018).

Protocol design and registration

The present study is a systematic review of the literature. The guiding research question was: "Which immature and adult species of the *Aedes* genus,

identified and collected in the field, tested positive for any arbovirus in the State of Minas Gerais?". This study was registered in the International Prospective Register of Systematic Reviews (Prospero, 2023) under registration number CRD42023475594. This systematic review adhered the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021).

Eligibility criteria

Observational studies developed in Minas Gerais described immature and adult species of *Aedes* genus that were identified and collected in the field with arboviruses and were considered eligible for this systematic review. The searches were conducted in October 2023 and updated until October 2024, considering studies published in Portuguese, English, and Spanish in scientific article format. There were no date restrictions for published studies. We excluded studies that did not correspond to the languages used in the search strategy, studies conducted in other States of Brazil or other countries, studies that were not in scientific article format (monographs, dissertations, theses, event summaries, editorials, and books) secondary studies, such as meta-analyses and literature reviews (narrative, integrative and systematic), and those that did not fit the study question.

Information sources and search strategies

The searches were carried out in the scientific electronic databases of *Biblioteca Virtual em Saúde* (BVS) in the complete collection of BVS, Scientific Electronic Library Online (SciELO), and the U. S. National Library of Medicine (PubMed), without restriction on the year of publication. These were done through search strategies using the Boolean operators "AND" and "OR". Each database used descriptors cataloged in the Descriptors em Ciências da Saúde (DeCS), and the Medical Subject Headings (MeSH) were used, considering the Portuguese, English, and Spanish languages. In the BVS and SciELO databases, the following combinations of terms were used: *Aedes* AND *Arbovírus* OR Arbovirus OR Dengue virus OR Chikungunya virus OR Zika virus AND Minas Gerais. For the PubMed database, the following combinations of terms were used: *Aedes* AND Arbovirus OR Dengue virus OR Chikungunya virus OR Zika virus AND Minas Gerais. Studies that contained the descriptors in the title, abstract, and keywords were selected.

Studies selection and data extraction

For the selection of studies and data extraction, after removing duplicated records, two independent researchers selected the articles by title, abstract, and full text, following the predefined inclusion and exclusion

criteria, meeting later to verify the inclusion and exclusion agreement. After these steps, information was extracted from the full texts (selected studies). The following data were extracted from the studies: author and year; location; period; identified and collected immature and adult species of *Aedes* genus; site of viral infection in the vector; method of identifying the presence of viral RNA within the vector; identified arboviruses (arbovirus/serotype); general positivity; collection area; and capture trap.

RESULTS

Selected studies

Five hundred and twenty-four studies were selected in the search in the three electronic databases. Forty-six were excluded due to duplication. During the screening, 478 studies were selected, of which 453 records were excluded because they did not address issues relevant to this review. Twenty-five articles were fully assessed for eligibility. Of these, 16 studies were excluded after full-text review. Thus, nine studies (Figure 1) were included in this systematic review, with studies carried out between 1993 and 2023 (Table 1).

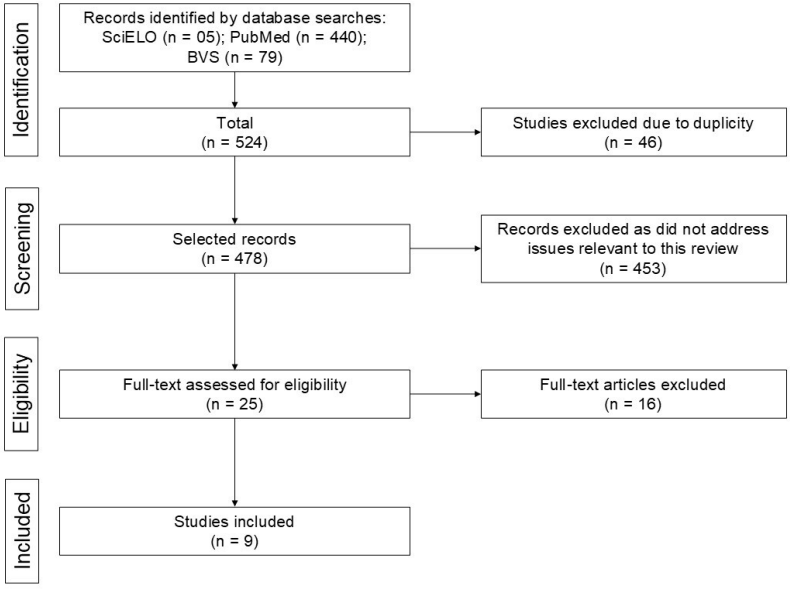

Figure 1. Flowchart of selected studies.

Table 1. Characterization of selected scientific studies on natural infection by arboviruses in immatures and adults of the *Aedes* genus in the State of Minas Gerais, Brazil.

SIN	Authors	Study location	Study period
1	Serufo et al. (1993)	Campos Altos	June 1993
2	Vilela et al. (2010)	Belo Horizonte	October 2005 to May 2006
3	Cecílio et al. (2015)	Ouro Branco and Ouro Preto	September 2011 to April 2013
4	Bezerra et al. (2016)	Belo Horizonte	2010 to 2014
5	Eiras et al. (2018)	Belo Horizonte	February to May 2016
6	Sedda et al. (2018)	Caratinga	August 2010 to July 2011
7	Andrade et al. (2022)	Contagem	January to June 2013
8	Cruz et al. (2023)	Itueta, Central de Minas, Alvarenga, São Sebastião do Maranhão, Itamarandiba, Caratinga and São Domingos das Dores	January 2016 to April 2017
9	Almeida-Souza et al. (2024)	Salinas	February to March 2023

SIN: study identification number.

Characterization of specimens of Aedes *genus regarding arbovirus positivity and collection process*

Among the nine selected studies, only four identified arboviruses in immatures (Serufo et al., 1993; Vilela et al., 2010; Cecílio et al., 2015; Andrade et al., 2022), with *Ae. aegypti* species being the most frequently collected. The species also had the highest number of adult specimens collected (n= 2,767; 72.0%).

Concerning the site of viral infection in the vector, two studies (Serufo et al., 1993; Sedda et al., 2018) did not describe the methodological procedure. However, in six studies (Vilela et al., 2010; Cecílio et al., 2015; Bezerra et al., 2016; Eiras et al., 2018; Andrade et al., 2022; Cruz et al., 2023), researchers macerated the entire bodies of the specimens. In a study (Almeida-Souza et al.,

2024), the mosquitoes were dissected into three parts: head, body, and legs. The bodies were initially tested, and the heads corresponding to the insects from the positive pools were also tested.

Concerning the method of identifying viral RNA, one study (Serufo et al., 1993) performed conventional polymerase chain reaction (PCR), two studies (Vilela et al., 2010; Cecílio et al., 2015) performed reverse transcription-polymerase chain reaction (RT-PCR) and six studies (Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Cruz et al., 2023; Almeida-Souza et al., 2024) performed real-time reverse transcription - polymerase chain reaction (RT-qPCR) (Table 2).

Regarding the arboviruses naturally identified in immatures and adults, most specimens were infected by DENV, as reported in seven studies (Serufo et al., 1993; Vilela et al., 2010; Cecílio et al., 2015; Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022), with confirmations for the different serotypes. Among these studies, one described positivity for ZIKV (Eiras et al., 2018) and one for CHIKV (Almeida-Souza et al., 2024). A study further described the natural infection by YFV (Cruz et al., 2023) (Table 2).

As for general positivity, the positivity rate for larvae belonging to the species *Ae. aegypti* was described in three (Vilela et al., 2010; Cecílio et al., 2015; Andrade et al., 2022), ranging from 0.18% to 32.1% for DENV virus. One study (Serufo et al., 1993) reported DENV-1 positivity in 2.1% of collected *Ae. albopictus* larvae. Regarding studies that addressed arbovirus positivity in adults, there was a variation from 5.5% to 16.9% for DENV-infected *Ae. aegypti*. Three studies (Bezerra et al., 2016; Sedda et al., 2018; Cruz et al., 2023) described positivity in adult specimens of *Ae. albopictus*, with records of natural infection by DENV (Bezerra et al., 2016; Sedda et al., 2018) and YFV (Cruz et al., 2023). Furthermore, one study showed CHIKV positivity in adult specimens of *Ae. aegypti* (Almeida-Souza et al., 2024) (Table 2).

Regarding the collection area, two studies (Cecílio et al., 2015; Cruz et al., 2023) did not describe the location where the specimens were captured. In six studies (Vilela et al., 2010; Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Almeida-Souza et al., 2024), specimen collections were carried out in urban areas, and one study (Serufo et al., 1993) was conducted in urban and peri-urban areas. Regarding the traps used, one study (Serufo et al., 1993) did not describe the collection method. The other studies used ovitraps and Pasteur nuggets to collect larvae and MosquiTRAP, BG-Sentinel, BG-GAT, Nasci aspirators, manual vacuum cleaners, and a hand-held entomological net to capture adults (Vilela et al., 2010; Cecílio et al., 2015; Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Cruz et al., 2023; Almeida-Souza et al., 2024) (Table 2).

Table 2. Description of the selected studies on species of immature and adult mosquitoes collected, identified arboviruses, and collection processes for specimens of the *Aedes* genus in the State of Minas Gerais, Brazil.

SIN	Authors	Species of immatures collected	Adult species collected	Site of viral infection in the vector	Molecular identification method	Arbovirus identified (arbovirus/ serotype)	General positivity	Collection area	Capture trap
1	Serufo et al. (1993)	*Ae. albopictus*: 100.0% (n=1,128; 93 pools)	*Ae. fluviatilis*: 10.4% (n=155; 4 pools)	NR	PCR	Dengue (DENV-1)	Larvae: *Ae. albopictus*: 2.1% (2 pools)	Urban and Periurban	NR
2	Vilela et al. (2010)	*Ae. aegypti*: (n=5,573; 101 pools)	*Ae. aegypti*: 100.0% (n=237; 25 pools)	Full body	RT-PCR	Dengue (DENV-3)	Larvae: *Ae. aegypti*: 0.18% (1 pool); Adults: *Ae. aegypti*: 16.9% (4 pools)	Urban	Ovitrap, MosquiTRAP version 2.0 and BG-Sentinel
3	Cecílio et al. (2015)	*Ae. aegypti*: 84,9% (n=945; 54 pools); *Ae. albopictus*: 15.1% (n=168)	NR	Full body	RT-PCR	Dengue (DENV)	*Ae. aegypti*: 7.4% (4 pools)	NR	Ovitrap

4	Bezerra et al. (2016)	NR	*Ae. albopictus*: 100.0% (n=511)	Full body	RT-qPCR	Dengue (DENV)	*Ae. albopictus*: 15.4% (n=79)	Urban	BG-Sentinel
5	Eiras et al. (2018)	NR	*Ae. aegypti*: 96.3% (n=1,506; 82 pools)	Full body	RT-qPCR	Dengue (DENV-1 and DENV-3) and Zika (ZIKV)	*Ae. aegypti*: 13.4% (11 pools)	Urban	BG-GAT
6	Sedda et al. (2018)	NR	*Ae. aegypti*: 89.7% (n=685); *Ae. albopictus*: 9.6% (n=74)	NR	RT-qPCR	Dengue (DENV-1, DENV-3 and DENV-4)	*Ae. aegypti*: 5.5% (n=38); *Ae. albopictus*: 6.7% (n=5)	Urban	MosquiTRAP
7	Andrade et al. (2022)	*Ae. aegypti*: 100.0% (28 pools)	*Ae. aegypti*: 100.0% (n=174)	Full body	RT-qPCR	Dengue (DENV-1, DENV-2, DENV-3 and DENV-4)	Larvae: *Ae. aegypti*: 32.1% (9 pools); Adults: *Ae. aegypti*: 8.6% (n=15)	Urban	Pasteur pipettes and BG-Sentinel

8	Cruz et al. (2023)	NR	*Ae.* spp: 11.5% (n=334; 45 pools)	Full body	RT-qPCR	Yellow Fever (YFV)	*Ae. albopictus*: 11.1% (5 pools); *Ae. scapularis*: 4.4% (2 pools); *Ae. argyrothorax*: 4.4% (2 pools); *Ae. serratus*: 4.4% (2 pools); *Ae.* sp.: 2.2% (1 pool)	NR	Hand entomological net
9	Almeida-Souza et al. (2024)	NR	*Ae. aegypti*: 100.0% (n=165; 31 pools)	Body and head	RT-qPCR	Chikungunya (CHIKV)	*Ae. aegypti*: 32.2% (n=53; 10 pools)	Urban	Nasci aspirators

Ae.: *Aedes*; sp: unidentified specie; spp: unidentified species; =: equal to; %: percentage; DENV: Dengue virus; YFV: Yellow Fever virus; ZIKV: Zika virus; PCR: polymerase chain reaction; RT-PCR: reverse-transcriptase - polymerase chain reaction; qPCR: real-time - polymerase chain reaction; RT-qPCR: real-time reverse transcription - polymerase chain reaction; NR: not reported; SIN: study identification number.

By relating the capture municipalities to the species found, it was possible to observe that, in most municipalities, *Ae. aegypti* and *Ae. albopictus* showed greater circulation. Furthermore, it was found that although most collected specimens were infected with DENV, YFV exhibited a broader distribution among municipalities with positive specimens (Figure 2).

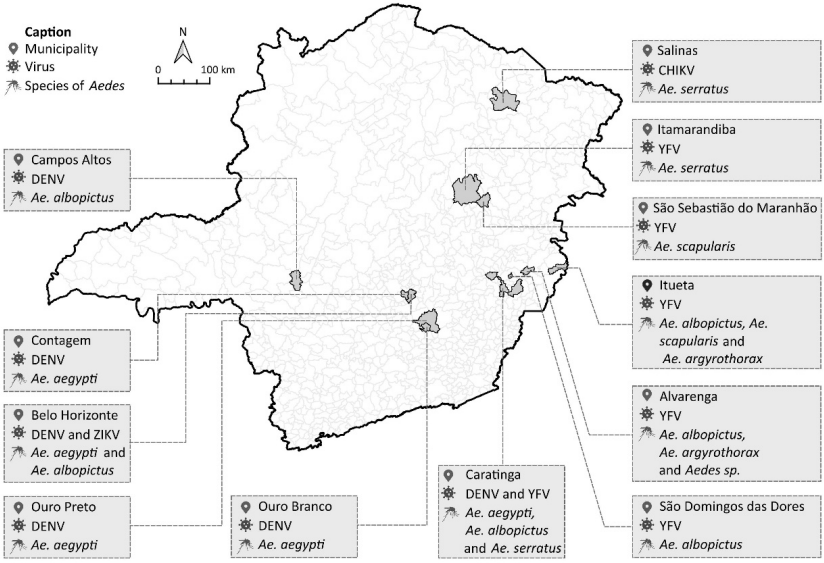

Figure 2. Distribution of *Aedes* mosquito species and relationship with arboviruses in Minas Gerais, Brazil. *Ae.*: *Aedes*; DENV: Dengue virus; YFV: Yellow Fever virus; CHIKV: Chikungunya virus; ZIKV: Zika virus.

DISCUSSION

This systematic literature review describes natural arboviruses infection in immature and adult mosquitoes of *Aedes* genus in Minas Gerais, Southeast Region of Brazil. The research was characterized through studies published in scientific literature, referring to the period from 1993 to 2024, carried out in the mesoregions of Triângulo Mineiro, Vale do Rio Doce, Sul de Minas, Metropolitan area of Belo Horizonte, Zona da Mata, and Jequitinhonha. It was verified through this systematic review that after the first records of autochthonous transmission of dengue in the State in 1987 in Pirapetinga (Sefuro et al., 1993), some studies sought to evaluate the arboviruses that naturally infect immatures and adults of *Aedes* genus, mainly from specimens of *Ae. aegypti*, given its vector capacity to transmit diseases other than dengue.

To our knowledge, no previous systematic review has been published on this topic for the State of Minas Gerais.

In addition to dengue, since the first confirmed cases of chikungunya in 2016 in Minas Gerais, the State has demonstrated a considerable increase in simultaneous dengue and chikungunya fever infections. The human co-infection rate is 11% (UFMG, 2023), demonstrating a high circulation of these arboviruses in immature and adult individuals of *Aedes* genus. Minas Gerais was the Brazilian State presenting the highest number of confirmed cases of dengue for 2023, with 312,592 cases of the 1,408,683 registered across the country. As for chikungunya, the State registered 77,887 confirmed cases of the 127,956 described throughout Brazil (SES-MG, 2023; MS, 2024). The State has tropical and semi-arid climates, which provide suitable conditions for the reproduction and survival of *Aedes* mosquitoes. Therefore, the high temperatures between December and March have a direct link to the greater larval infestation by adults of *Ae. aegypti* in cities across the State (Fonseca & Garcia, 2022), as well as for the infection and transmission of different arboviruses.

Out of four studies that sought to identify arboviruses in immature forms (Serufo et al., 1993; Vilela et al., 2010; Cecílio et al., 2015; Andrade et al., 2022), *Ae. aegypti* species represented the highest number of collected larvae. Five studies (Vilela et al., 2010; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Almeida-Souza et al., 2024) also described a greater number of adult specimens of *Ae. aegypti* (n= 2,767; 72.0%) among all collected species. Regarding the positivity rate for larvae belonging to the species *Ae. aegypti*, only three studies (Vilela et al., 2010; Cecílio et al., 2015; Andrade et al., 2022) described data for Minas Gerais, with a variation from 0.18% to 32.1% of DENV positivity. One study (Serufo et al., 1993) reported DENV-1 positivity in 2.1% of *Ae. albopictus* larvae collected. In the study developed by Medeiros et al. (2018) in Natal, Rio Grande do Norte, Northeast Region of the country, DENV-4 infection was described in immature *Ae. aegypti*; however, there was no detection of DENV in immature *Ae. albopictus*.

Regarding arbovirus positivity in adults, there was a variation from 5.5% to 16.9% for female *Ae. aegypti* specimens naturally infected by DENV (Vilela et al., 2010; Sedda et al., 2018). On the other hand, a study carried out in the city of São Paulo, Southeast Region of Brazil, between 2014 and 2016, described for the first time the natural vertical transmission of DENV in male *Ae. albopictus* mosquitoes infected by DENV-3 (Ferreira-de-Lima et al., 2020). The study points to the detection of DENV-3 in mosquitoes in years when there were no autochthonous human cases, suggesting the silent circulation of this serotype and indicating that green areas may be maintaining serotypes that are not circulating in humans, probably through a vertical transmission mechanism (Ferreira-de-Lima et al., 2020). However, in 2020 and 2023, recent autochthonous transmission of DENV-3 in humans was observed, indicating a

silent spread of the virus in São Paulo (Fujita et al., 2024). This fact highlights the importance of conducting more entomological surveillance studies to prevent the occurrence of new cases and potential epidemics.

Both immature and adult forms of different *Aedes* species were naturally positive for arboviruses, mainly being infected by DENV (Serufo et al., 1998; Vilela et al., 2010; Cecílio et al., 2015; Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022). A study carried out between 2011 and 2014 in Natal, Rio Grande do Norte, Northeast Region of Brazil, described that around 89.0% of the 1,333 immature insects collected and 95.0% of the 1,360 adult females captured belonged to the *Ae. aegypti* species, of which 20.7% were naturally infected by DENV (Medeiros et al., 2018). Furthermore, a study conducted in four municipalities in Mato Grosso, Central-West Brazil, between 2017 and 2018 highlighted that 27.3% of the 1,139 adult *Ae. aegypti* specimens tested positive for various viruses. These included not only DENV but also CHIKV, ZIKV, YFV, Eastern Equine Encephalitis virus (EEEV), Oropouche virus (OROV), and Ilheus virus (ILHV) (Ferreira et al., 2020).

One study (Eiras et al., 2018) described natural ZIKV infection in adult specimens of *Ae. aegypti* in Belo Horizonte, the capital of the State. Another research conducted from 2017 to 2020 in Foz do Iguaçu, Paraná, South Region of Brazil, demonstrated that three (1.3%) of the 221 pools of *Ae. aegypti* adults collected in urban areas were also positive for ZIKV (Leandro et al., 2022). The authors emphasize that locating naturally infected vectors provides a support tool for local health managers to prioritize intervention areas to prevent outbreaks caused by DENV, CHIKV, and ZIKV (Leandro et al., 2022). One study (Cruz et al., 2023) described natural infection by YFV in adult mosquitoes of the *Aedes* genus in municipalities situated in the Vale do Rio Doce, Zona da Mata, and Jequitinhonha mesoregions. A study conducted in 2017 in five municipalities of Espírito Santo, a neighboring State of Minas Gerais, reported the first YFV infection in field-captured *Ae. aureolineatus* (1.2%). This finding suggests that mosquitoes of this species may play a secondary role in arbovirus transmission (Stanzani et al., 2022).

CHIKV infection in adult specimens of *Ae. aegypti* was described in one study (Almeida-Souza et al., 2024). In a survey conducted in Codó, Caxias, São Mateus do Maranhão, and Alto Alegre do Maranhão, located in the State of Maranhão, Northeast Brazil, between 2016 and 2017, CHIKV positivity was observed in 1.9% of the 348 adult *Ae. aegypti* specimens (Aragão et al., 2019). Also worth noting is a study conducted at Parque das Dunas, in Natal, the capital of Rio Grande do Norte, in 2018: CHIKV was identified in females of *Ae. aegypti*, *Ae. albopictus* and *Ae. fluviatilis* and in males of *Ae. albopictus* (Ximenes et al., 2020).

Other potential species that could act as vectors for arbovirus transmission were also described in the included studies. As for specimens of

Ae. albopictus, one study (Serufo et al., 1993) addressed DENV infection in larvae, two studies (Bezerra et al., 2016; Sedda et al., 2018) described DENV infection in adults, and one study (Cruz et al., 2023) showed that adults of this species were naturally infected by YFV. The species *Ae. albopictus*, *Ae. scapularis*, *Ae. argyrothorax*, and *Ae. serratus* were described in one study (Cruz et al., 2023) with natural YFV infection. A survey conducted in the States of São Paulo, Rio de Janeiro, and Minas Gerais between 2015 and 2018 documented natural YFV infection in specimens of *Ae. scapularis* and *Ae. taeniorhynchus*, with positive specimens captured in Maricá, Rio de Janeiro. The authors point out that these species may play a secondary role in YFV transmission in the State (Abreu et al., 2019).

Regarding the site of viral infection, two studies (Serufo et al., 1993; Sedda et al., 2018) did not describe the methodological procedure adopted. To this end, in six studies (Vilela et al., 2010; Cecílio et al., 2015; Bezerra et al., 2016; Eiras et al., 2018; Andrade et al., 2022; Cruz et al., 2023), the researchers used maceration of the entire body of the specimens. In a study carried out in Manaus, Amazonas, North Region of Brazil, between 2018 and 2021, researchers also macerated the entire body of the specimens while searching for specimens naturally infected by arboviruses (Gomes et al., 2023). In a study by Almeida-Souza et al. (2024), only the bodies of the mosquitoes were initially tested. Subsequently, when a pool of bodies tested positive, the heads of the mosquitoes were also tested. In turn, in a study conducted in three municipalities in the State of Sergipe, Northeast Region of the country, in 2018, the authors sectioned the captured specimens, and only the heads and thoraxes were used to detect arboviruses (Jesus et al., 2022).

Viral RNA identification methods were described in one study (Serufo et al., 1993) using PCR; in two studies (Vilela et al., 2010; Cecílio et al., 2015) using conventional RT-PCR; and in six studies (Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Cruz et al., 2023; Almeida-Souza et al., 2024) using RT-qPCR. The use of different viral RNA identification techniques is due to several factors, such as the extension of time for providing conventional RT-PCR results and the lower risk of contamination, as well as the generation of quantitative results with greater precision through RT-qPCR (SPLabor, 2023). The RT-PCR technique is widely used for viral identification in mosquitoes; however, poor conservation of the material can compromise the integrity of the RNA and lead to false-negative results (Barbosa, 2016).

Concerning the specimen collection area, six studies (Vilela et al., 2010; Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Almeida-Sousa et al., 2024) conducted the capture in urban areas, and one study (Serufo et al., 1993) in both urban and peri-urban areas in the State of Minas Gerais. Most studies are carried out in urban environments due to the urban habits of *Ae. aegypti* and its epidemiological importance being the

primary vector of dengue fever, chikungunya fever, Zika, and urban yellow fever (Sousa et al., 2021).

Regarding the capture method, only one study (Serufo et al., 1993) did not describe the collection object. Studies with immature insects used ovitraps and Pasteur pipettes to collect specimens (Vilela et al., 2010; Cecílio et al., 2015; Andrade et al., 2022). There is evidence that oviposition traps can estimate the infestation rate without requiring inspection of water containers and breeding sites. Thus, ovitraps have been proposed as an alternative tool for monitoring *Ae. aegypti* and *Ae. albopictus* in urban areas (Moura et al., 2020). Studies conducted with adults used MosquiTRAP, BG-Sentinel, BG-GAT traps, Nasci aspirators, manual vacuum cleaners and a hand-held entomological net to capture these (Vilela et al., 2010; Bezerra et al., 2016; Eiras et al., 2018; Sedda et al., 2018; Andrade et al., 2022; Almeida-Souza et al., 2024). MosquiTRAP traps are widely used because they attract females of *Ae. aegypti* during the oviposition period through visual and olfactory stimuli. BG-Sentinel traps attract adults of different physiological stages as they imitate human convection currents (Degener et al., 2014).

Considering the results obtained from the studies included in this systematic review, there is an urgent need for more effective strategies to combat *Aedes* mosquitoes. These measures should focus especially on *Ae. aegypti* surveillance and control, as the species is well adapted to urban areas and is the main responsible for the transmission of dengue, chikungunya, Zika virus, and yellow fever in urban areas. However, with the confirmation of natural infection in specimens of *Ae. albopictus*, the importance of secondary transmission of some arboviruses by these vectors should not be overlooked and actions to prevent the urbanization of this species should be considered. Furthermore, considering the reported positivity of *Ae. scapularis*, *Ae. argyrothorax* and *Ae. serratus,* more studies investigating their vector competence should be encouraged, given their potential secondary role in the transmission of arboviruses in the State of Minas Gerais.

This systematic review includes important data on natural infection in immatures and adults of the *Aedes* genus in Minas Gerais but has limitations. There was a lack of studies since, for 31 years, only nine studies conducted on the topic were found by the established study search criteria. Among the selected studies, only four analyzed natural infection in immature specimens, thus limiting knowledge regarding the transovarian transmission of arboviruses.

Considering this systematic review, it is possible to conclude that specimens of *Ae. aegypti*, whether in immature or adult forms, had a more number of specimens captured, in addition to having the highest percentage of natural arbovirus infection among the other species collected in Minas Gerais. Most specimens analyzed were naturally infected by DENV, with varied serotypes across different studies. Furthermore, most of the specimens in the included studies were collected in urban areas, using different traps,

such as ovitraps, BG-Sentinel and hand-held entomological nets. Therefore, new studies are needed to investigate the secondary vector competence of other species within the *Aedes* genus, considering the detection of various arboviruses across different species in the State.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest to disclose.

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