Multidisciplinary approach for surveillance and risk identification of yellow fever and other arboviruses in Colombia

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ABSTRACT

Arbovirus, a critical threat to human health, have complex and dynamic life cycles. With reports of Yellow fever virus (YFV) causing spillover from sylvatic transmission cycles, and dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses expanding from urban to rural areas. We explored a multidisciplinary approach to analyze arbovirus transmission through vectors, and identify biological and sociodemographic determinants associated with their transmission risk in urban and rural areas in a Colombian municipality. We visited 178 urban and 97 rural households, registered sociodemographic characteristics and vaccination status for each of these households, collected adult and immature mosquitoes at the intra-, *peri*-, and extra-domicile, and surveyed forest patches in rural areas. Infections of YFV, DENV, ZIKV, and CHIKV in the mosquitoes collected in the wild were analyzed using a reverse transcriptase PCR. We identified various risk factors of transmission associated with a high *Aedes aegypti* infestation in urban areas and their presence in rural settlements and *Haemagogus janthinomys* and other sylvatic mosquitoes near urban areas. The collected *Ae. aegypti* females from urban areas had a high infection rate of YFV (5.8%) and CHIKV (58.8%), and those from rural settlements had a high infection rate of DENV (33%), CHIKV (16.7%), and ZIKV (16.7%). The infection rates of YFV in the thorax of the sylvatic mosquitoes *H. janthinomys* and *Aedes serratus* collected from the forest patches were 14.3 and 42.1%, respectively. We could discern the transmission determinants associated with climatic, socioeconomic, and anthropogenic factors and YFV vaccination status. This multidisciplinary approach for surveillance of arboviral diseases allowed us to independently detect and integrate factors indicating an early risk of rural transmission of DENV, CHIKV, and ZIKV and rural and urban outbreaks of YFV in the study area. This study provides a helpful tool for designing and focalizing prevention strategies.

1. Introduction

Arboviruses present a significant threat to human health, specifically in tropical and subtropical regions, causing extensive morbidity and mortality [1]. In America, dengue (DENV), Zika (ZIKV), chikungunya (CHIKV), and yellow fever (YFV) viruses have similar urban transmission cycles, with *Aedes aegypti* being the primary vector. YFV is maintained in sylvatic cycles involving non-human primates and sylvatic vectors (*Haemagogus* and *Sabethes*) [2]. However, a recent increase of YFV periurban outbreaks is reported in South America, despite the presence of an approved vaccine [3–5]. Furthermore, the transmission

of DENV, ZIKV, and CHIKV is expanding its limits to rural settlements [6,7].

Arbovirus transmission is mediated through susceptible vectors (urban or sylvatic mosquitoes) and their ecology, which is affected by climatic and anthropogenic factors [8,9]. Arbovirus detection in mosquitoes is crucial for understanding transmission dynamics, surveillance, and prevention strategies [10]. the socio-demographic characteristics of local communities, and their misperceptions and misconceptions, need to be understood and addressed to engage the population in preventive behavior [11], in this sense a multidisciplinary approach must be implemented to understand the transmission

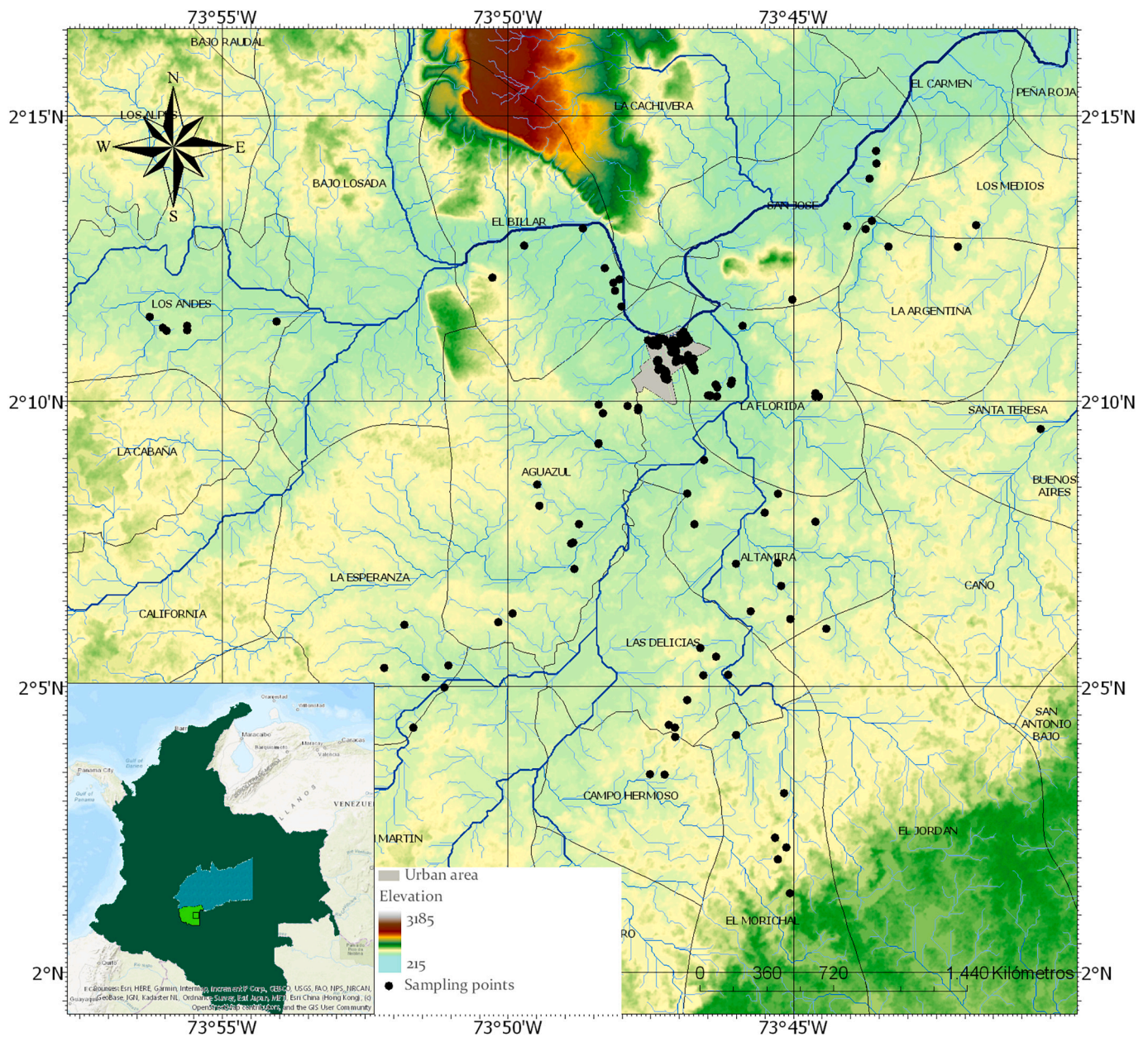**Fig. 1.** Map of the study area shows the location of El Meta department (teal) in Colombia and the La Macarena (lime) municipality. Sampling points are shown as black dots, the urban area is in gray, and the names of the sampled and surrounding rural settlements are given in bold black letters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dynamics [12].

The ecological, biological, demographical, socio-economical, and epidemiological characteristics of La Macarena municipality, facilitate arboviral transmission [13,14]. Factors increasing the risk of changes in arboviral transmission dynamics in the region include international tourism, the extraction of natural resources, and extending the agricultural borders to sylvatic areas, activities amplified after peace agreements [15]. This study explored a multidisciplinary approach to analyze arbovirus transmission through vectors, and identify biological and sociodemographic determinants associated with their transmission risk in urban and rural areas in a Colombian municipality.

2. Methods

2.1. Study area

We conducted visits between August and November 2019 in an urban area and eight rural settlements in La Macarena municipality, Meta, Colombia (Fig. 1), located at 2° 10′ 48″ N and 73° 46′ 49″ W, 233 m above sea level. The municipality presents a bimodal rainfall pattern, a dry season from December to May, a rainy peak between June and November, with ~2914 mm annual rainfall, 86% relative humidity, and 25 °C temperature [13].

2.2. Sample size

A cross-sectional study was conducted in which the unit of analysis was the household of each person who agreed to participate. The sample

Table 1
Culicidae immature stages collected in urban and rural areas of La Macarena (Meta), Colombia.

	Species	Location*	Immature Stages		Total
			Larvae	Pupae	
Area	Aedes aegypti	Intra	889	407	1296
	Culex quinquefasciatus	Intra	10	7	17
	Culex spp.	–	103	2	105
	Toxorhynchites sp.	–	1	–	1
	Total Urban		1003	416	1419
Rural	Aedes aegypti	Intra	12	27	39
	Aedes spp.	–	–	6	6
	Coquillettidia nigricans	Extra	–	1	1
	Culex coronator	Extra	–	3	3
	Culex quinquefasciatus	Peri, Extra	11	31	42
	Culex urichii	Extra	71	6	77
	Culex spp.	–	38	60	98
	Haemagous janthinomys	Extra	10	3	13
	Limatus asulleptus	Extra	–	14	14
	Limatus durhamii	Extra	39	34	73
	Orthopodomyia spp.	–	–	4	4
	Psorophora albipes	Extra	–	2	2
	Psorophora ferox	Peri	–	1	1
	Sabethes cyaneus	Extra	3	–	3
	Sabethes quasicyaneus	Extra	17	2	19
	Trichoprosopon spp.	–	3	3	6
	Trichoprosopon digitatum	Extra	77	80	157
	Toxorhynchites spp.	–	18	–	18
	Wyemoyia spp.	–	11	–	11
	Total Rural		310	277	587

* Intra-domicile (Intra), peri-domicile (peri), forest patches at the extra-domicile (extra).

size was obtained using projected population data from the census [16]. The sample size was determined with SSPropor (http://www.openepi.com/). We sampled 178 urban and 97 rural households (Fig. 1), each house was visited once, and sampling was conducted daily.

2.3. Permits, and ethical considerations

Before data collection, a written informed consent was obtained from the inhabitants, as approved by the Comité Institucional de Ética en Investigaciones of Universidad El Bosque, Bogotá, Colombia (Acta No 006–2019, March 14, 2019). Sampling was authorized by the Autoridad Nacional de Licencias Ambientales (resolution number 01470, November 17, 2017).

2.4. Sociodemographic data collection

Participants were enquired about different sociodemographic, and economic characteristics of their households, vaccination status, the ability to recognize, and report urban and sylvatic vectors or non-human primates near the household. Collected information is presented in the Table S1.

2.5. Mosquito collection

Immature mosquitoes were collected in artificial and natural water-holding containers in three settings: intradomicile, *peri* (0–10 m from household), and extra-domicile (forest patches located 10–500 m from household). Pupae were allowed to emerge. The following characteristics of the breeding sites were recorded: materials (clay, ceramic, concrete, glass, metal, organic, plastic, and rubber), sunlight exposure (uncovered, partial, or covered), setting (intra-, peri-, or extra-domicile), structure (permanent or temporary), area (urban or rural), and whether inhabitants reported a control strategy (chemical, physical, biological or none).

Adult resting mosquitoes were captured using a Prokopack aspirator [17] in the intra- and peri-domicile areas for 15 min each, while those in

forest path areas were captured using entomological nets. Additionally, we used two CDC miniature light trap (Curtis Dyna-Fog's Ltd., http://www.dynafog.com/) and four BG-sentinel traps baited with BG-Lure (Biogents, https://eu.biogents.com/). GPS coordinates were obtained using Garmin GPS 62 s.

2.6. Mosquito identification

Taxonomic identification of immature and adult mosquitoes was carried out under a stereo microscope Stemi ™ DV4 (Zeiss, https://www.zeiss.com/microscopy/), using different taxonomic keys [18–20]. Representative mosquitoes of each species were deposited in the Science Museum of the Universidad El Bosque (MCUB-R-HE-DI-000518 and MCUB-R-HE-DI-000561). Once identified, 2–30 *Ae. aegypti* individuals were pooled by developmental stage, sex, date, and locality and preserved in RNAlater® (Thermo Fisher Scientific, Waltham, Massachusetts U.S.A). For the females of *Haemagogus janthinomys* and *Aedes serratus*, the abdomen and head-thorax regions were separated using micro-scissors and preserved individually in RNAlater®, whereas males and immature mosquitoes were processed similarly to *Ae. aegypti*.

2.7. RNA extraction and amplification

RNA extraction was performed using the RTP® DNA/RNA Virus Mini Kit (Stratec, Birkenfeld, Germany) based on the protocol provided by the manufacturer, and quantified using a NanoPhotometer® NP80 (Implen, Munich, Germany). Viral RNA retro-transcription and amplification were performed using the Luna® Universal kit One-Step RT-PCR (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A), with 400 ng of purified RNA, and a multiplex nested RT-PCR procedure was performed. For YFV, we used the primers designed in our lab YFUTRF- GCTAATTGAGGTGYATTGGTC and VFAR-CGAACTCCTCGTCGTACCATA (outer primers) and VFAF-CAAATCGAGTTGCTAGGCAAT and VFAR-CGAACTCCTCGTCGTACCATA (inner primers), to amplify a 145 bp fragment between positions 36 and 181 in the YFV genome. For DENV, CHIKV, and ZIKV, we used the protocol and primers previously described [21]. Culture-harvested viruses were used as controls.

The final amplification products were separated on 2% agarose gel stained with ethidium bromide. Positive YFV samples were sequenced and aligned using Mega X [22], with representative sequences from BLAST® analysis (NCBI, https://blast.ncbi.nlm.nih.gov/). The final alignment involved 29 nucleotide sequences of 134 bp. Genetic distance was calculated using the Kimura 2-parameter method, with an unrooted phylogenetic tree constructed using neighbor-joining.

2.8. Entomological indices

We used the following *Aedes* indices: house index (HI), container index (CI), Breteau index (BI), and pupae-per-person index (PPI) as described by the CDC [23]. Female *Ae. aegypti* infestation was estimated as the number of mosquitoes collected over 15 min with one Prokopack trap in the intra-domicile. We calculated the infection rate (IR) using the Firth estimator for unequal pool sizes, using the function PooledBin in the BinGroup R package in R program 3.5.2 [24], and compared the percentage of positive samples with those of other studies.

2.9. Environmental variables and spatial analysis

The average, minimum and maximum temperature, and total precipitation were obtained from WorldClim [25]. The normalized differenced vegetation index (NDVI) was estimated using Sentinel 2B satellite images. The entomological indices CI and PPI were projected, and extrapolated using inverse distance weighting in ArcGis Pro 2.8.3.

Table 2

Culicidae adults collected with different traps in urban and rural areas of La Macarena (Meta), Colombia.

Area	Species	Location*	BG-sentinel		CDC		Entomological net		Prokopack		Total
			Female	Male	Female	Male	Female	Male	Female	Male	
Urban	*Aedeomyia squamipennis*	Peri			1						1
	Aedes aegypti	Intra, Peri	10	5		1			305	239	560
	Aedes fulvithorax	Peri							2		2
	Aedes hastatus	Intra								1	1
	Aedes hortator	Intra								1	1
	Anopheles marajoara	Intra, Peri			3				1		4
	Anopheles nimbus	Intra							2		2
	Anopheles spp.	–				1					1
	Culex quinquefasciatus	Intra, Peri	10	61	9	11			335	532	958
	Culex spp.	–			12			1	14	1	28
	Psorophora dimidiata	Intra, Peri			2						2
	Uranotaenia geometrica	Intra								1	1
	Uranotaenia hystera	Peri							1		1
	Uranotaenia lowii	Intra, Peri			1				1		2
	Total Urban		20	66	28	13	0	1	661	775	1564
Rural	*Aedes aegypti*	Intra							11	12	23
	Aedes angustivittatus	Extra					4	1		1	6
	Aedes fulvus	Extra					11	3			14
	Aedes hortator	Extra					1				1
	Aedes scapularis	Extra						1			1
	Aedes serratus	Intra, Extra					417	18	4		439
	Aedes spp	–			1		1	8	2		12
	Anopheles marajoara	Extra					1				1
	Anopheles nimbus	Intra, extra					153	7	1		161
	Anopheles oswaldoi	Extra			2						2
	Anopheles triannulatus	Extra			1						1
	Coquillettidia spp	–					1				1
	Culex (melanoconion) sp.	–					1				1
	Culex coronator	Extra						1			1
	Culex quinquefasciatus	Intra, peri			6		26	22	665	1101	1820
	Culex spp.	–					29	3	15	3	50
	Culex urichii	Extra					1				1
	Haemagous janthinomys	Extra					46	2			48
	Jhonbelkinia longipes	Extra					1				1
	Jhonbelkinia sp.	–						1			1
	Limatus asulleptus	Peri, extra	1				15		1	1	18
	Limatus durhamii	Intra, peri, extra					10		2		12
	Limatus spp.	–					16				16
	Psorophora albipes	Intra, extra	1				367	8	2		378
	Psorophora cingulata	Extra					1				1
	Psorophora dimidiata	Extra					1				1
	Psorophora ferox	Extra					82	1			83
	Psorophora spp.	–					1		2		3
	Sabethes belisarioi	Extra							1		1
	Sabethes chloropterus	Extra					3				3
	Sabethes cyaneus	Extra					1				1
	Sabethes quasicyaneus	Extra					2				2
	Sabethes spp.	–					6				6
	Sabethes tarsopus	Extra					2				2
	Sabethes tridentatus	Extra							2		2
	Sabethes undosus	Extra					3				3
	Trichoprosopon digitatum	Intra, extra	17				77	28	1		123
	Trichoprosopon spp.	–	1				1	1	1		4
	Wyeomyia personata	Peri							1		1
	Wyeomyia spp.	–					15		5		19
	Total Rural		20	0	10	0	1296	105	716	1118	3264

* Intra-domicile (Intra), peri-domicile (peri), forest patches at the extra-domicile (extra).

2.10. Statistical analysis

The presence of immature *Ae. aegypti* was analyzed based on breeding site characteristics, using a generalized linear model (GLM) with binomial distribution using the MASS package in R program version 3.5.2. The female mosquito density was analyzed using a GLM with Poisson distribution. The predictor variables were: the number of house inhabitants, piped water supply and its periodicity, the primary source of water for drinking, water storage conditions, waste recollection service, waste disposal in the household, if received information on yellow fever, the source of information, if yellow fever mosquito spotted inside the house, the type of dwelling). The environmental variables were precipitation, elevation, normalized difference vegetation index, distance to the nearest river, temperature range, maximum, medium and minimum temperatures. The presence of multicollinearity was assessed using the generalized variance inflation factor with the "car" package.

3. Results

3.1. Sociodemographic characteristics

We visited 275 households inhabited by 966 residents, with 478 (49%) men and 488 (51%) women. Several differences existed between urban and rural areas associated with dwelling type, drinking water
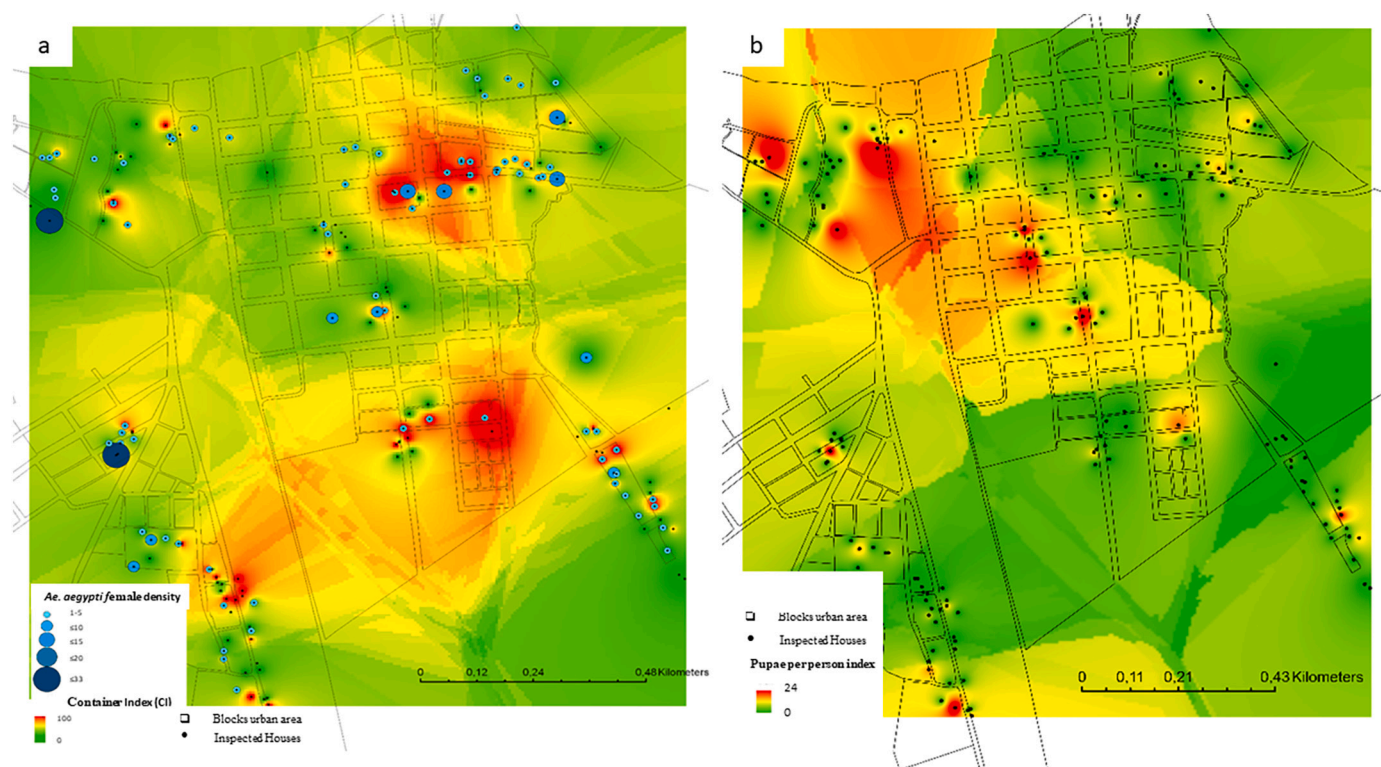
Fig. 2. Heat maps of some entomological indexes (a) Heat map of the container index (CI) for *Ae. aegypti* at the urban area, extrapolated for the whole surface based on the results for the inspected houses (black dots). The collected *Ae. aegypti* females are also represented by blue circles (b). Heat map of pupae-per-person index (PPI) for *Ae. aegypti* in the urban area, extrapolated for the whole surface based on the results for the inspected houses (black dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sources, public services, and waste disposal (Table S1). For example, the most common option for storing water was a container with a lid (14.2% rural, 46.5% urban), and the most reported frequency of washing the containers was once a week (Table S1).

Of the household members, 75.9% affirmed to have received the yellow fever vaccine (Table S1). However, the vaccination status of 82.9% of these members remained uncorroborated. The primary source of information on yellow fever were health sector institutions and other sources such as radio and the local airport (Table S1). Most participants (63.6%) had not spotted monkeys near their houses. *Alouatta seniculus* (20% rural, 2.3% urban) was the most reported monkey species near their households. Only 12 participants had seen the corpses of monkeys; of these, seven (2.2% rural, 0.4% urban) and five (0.4% rural, 1.5% urban) had seen monkeys near and far from the household, respectively (Table S1).

3.2. Entomological survey

3.2.1. Immature stages

Of the 776 water-holding containers inspected, 298 (38.4%) [210 (70.4%) in urban areas and 88 (29.5%) in rural areas] were positive for the presence of Culicidae larvae or pupae. In total, 2006 immature individuals were collected (1313 larvae and 693 pupae). Eleven genera and thirteen species were identified, with a higher species richness associated with forest patches in rural areas (Table 1). *Ae. aegypti* and *Trichoprosopon digitatum* were the most encountered species in urban and rural areas, respectively (Table 1). Immature stages of *H. janthinomys* (ten larvae and two pupae) were found only in two tree holes in forest patches of two rural settlements.

In the urban areas, CI was 35% (166/473), HI was 52% (92/178), and BI was 89.9. In rural areas, CI was 2% (6/303), HI was 4% (4/103), and BI was 5.8. CI and PPI in the urban area displayed a differential

spatial pattern (Fig. 2). Wash basin was the most common positive breeding place in urban (37.5%) and rural (50%) areas (see Fig. 3). The GLM model could explain the prevalence of immature *Ae. aegypti* (24%); the significant variables were urban areas, households that did not report any larvae for control measures, and breeding sites in concrete or rubber (Table S2).

3.2.2. Adults

A total of 4828 adult mosquitoes were collected from urban and rural areas, corresponding to 13 genera and 36 Culicidae species (Table 2). Among the six genera and twelve species identified in the urban areas, *Culex quinquefasciatus* was the most common, followed by *Ae. aegypti* (Table 2). Among the 11 genera and 30 species collected in the rural areas, *Cx. quinquefasciatus* was the most frequent, followed by *Ae. serratus* (Table 2). *Ae. aegypti* was found in both urban and rural areas (Supplementary Fig. S1), whereas the sylvatic YFV vectors, *H. janthinomys* and *Sabethes chloropterus* were prevalent close to urban areas (Supplementary Fig. S1). *Ae. serratus* mosquitoes were prevalent in intra- and extra-domicile settings in rural areas (Table 2).

3.3. Determinants for female infestation

A 40.7% of *Ae. aegypti* female infestation, was positively associated with the water sources in households having information about yellow fever, those that burnt or buried waste, and those with a high number of potential breeding places (Table 3). Of the environmental variables, temperature and precipitation were associated with female *Ae. aegypti* abundance (Table 3). There was not enough data on the infestation of *H. janthinomys* to conduct a statistical analysis.

Table 3
The generalized linear model (GLM), using a Poisson distribution, to assess *Ae. aegypti* female infestation against environmental and sociodemographic variables.

Predictor	Category	Estimate	Std. Error	Z value	P-value*
Intercept		−0.001	0.002	−0.5	0.6
Number of People living in the household		0.02	0.03	0.8	0.4
Household with piped water supply		0.09	0.2	3.5	**0.0004**
Periodicity of the piped water supply	Permanent	0.8	0.4	2.0	**0.04**
	Scheduled	0.6	0.3	1.8	0.05
primary source of water for drinking	Piped water	0.4	0.9	0.4	0.6
	Well, borehole	0.1	0.9	1.1	0.2
	River, stream	1.8	1.0	1.8	0.07
	Rain water	4.2	1.1	3.6	**0.0003**
Water storage conditions	Deposit or container with lid	−0.8	0.2	−4.4	**0.0000**
	Do not store water	−0.8	0.2	−3.6	**0.0003**
Previously received information on yellow fever		0.3	0.1	2.2	**0.02**
Yellow fever mosquito spotted inside the house		−0.03	0.01	−0.2	0.8
Type of dwelling	Room	0.9	0.003	0.008	0.9
	House	0.2	0.002	0.008	0.9
	Cabin	0.2	0.002	0.008	0.9
	Hut	0.2	0.002	0.008	0.9
Waste disposition	Deliver to waste collection service	0.02	0.07	0.02	0.9
	Throw it to a patio, plot, or empty land	−0.1	0.05	−0.03	0.9
	No answer	−0.1	0.03	−0.005	0.9
	Dispose it (burn or bury)	1.4	0.2	7.0	**0.0000**
Number of potential breeding places in the household		0.05	0.02	2.1	**0.03**
NDVI**		−0.7	1.2	−0.6	0.5
Distance to the nearest river or stream		−0.001	0.0004	−1.1	0.2
Precipitation		−0.09	0.04	−2.0	**0.04**
DTR***		−7.1	1.0	−7.0	**0.0000**
Medium temperature		0.6	0.1	5.3	**0.0000**

* Significant *P* values are in bold.
** Normalized difference vegetation index.
*** Diurnal temperature range.

3.4. Arbovirus detection

The IR was higher in females (IR: 19.4%) than in males (IR: 8.9%) in urban areas, in contrast to the pattern observed in rural areas (Fig. 4 a, b). The IR of YFV in *H. janthinomys* and the thorax and abdomen of *Ae. serratus* females was 65.1, 16.5, and 49%, respectively (Table 4 and Fig. 4c,d). High-quality viral RNA obtained from *Ae. aegypti* males and larvae and RNA isolated from the thorax and abdomen of *Ae. serratus* collected from a rural area yielded the same sequence (accession number ON505821). The consensus sequence belonged to the group of Central African and vaccine strains; a few reportedly could infect *Ae. aegypti* under the experimental conditions (Fig. 5). The consensus sequence had

a genetic distance of 3.6–5.1% from the American strains (Table S2) isolated from vertebrates and mosquitoes (Fig. 5).

In *Ae. aegypti* females (collected and reared), the highest IR was for CHIKV (190.3%), followed by YFV (37.6%), DENV (22.6%), and ZIKV (14.7%). CHIKV showed a high IR in both females (309.8%) and males (89.4%) from urban areas, whereas, in rural *Ae. aegypti* mosquitoes, DENV had the highest IR among females (77.7%). Only DENV3 was detected in the urban areas, whereas DENV2 and DENV3 were detected in the rural areas (Table 4). Unexpectedly, *H. janthinomys* had the highest IR for CHIKV (126.24) (Fig. 5c), followed by YFV (65.1) and DENV (47.7). All four arboviruses could infect *Ae. serratus*. Notably, the lowest infection rates occurred in the thorax than in the abdominal pools for the viruses (Fig. 4d).

4. Discussion

This study presents a novel integrated approach for the surveillance of arboviral diseases with different ecological transmission dynamics, allowing the identification of transmission risks at the local scale. Although most studies have focused on determining the abundance of immature or adult mosquitoes alone [26–29]; our findings highlight the importance of evaluating immature and adult infestation together. Modeling the spatial distribution of these indices and breeding site characteristics is valuable for designing and focusing on control strategies at fine scales, as evident from our model [30,31], furthermore the observed spatial pattern for immature stage presence was probably associated with breeding places availability and characteristics as was found by our model. This information helps to prioritize interventions and resources for mosquito control mainly because the detected *Ae. aegypti* infestation was higher than the limit proposed to avoid YFV urbanization [32]. The presence of *Ae. aegypti* in rural areas represents an additional risk for the rural and peri-urban transmission of different arboviruses. Entomological and virological surveillance by local health authorities must cover these areas.

The high mosquito richness in the study area was probably associated with modifications in landscapes, e.g., forest patches [33–35] favoring the proximity of sylvatic vectors to humans. The proximity of forest patches with high mosquitoes richness to urban areas and tourist spots in rural settlements increases the transmission risk of known and novel arboviruses with zoonotic potential. Reports of *Alouatta seniculus* near households as a risk factor for YFV transmission are associated with landscape modifications and wildlife captivity [3]. These results call for strengthening arbovirus surveillance and YFV vaccination measures to avoid a public health problem due to the risk that the local community and visitors are exposed. La Macarena represents an attractive ecotourism destination for travelers and economic activities favoring large-scale deforestation, including logging to expand the agrarian frontier and cocoa growth [36]. However, it is necessary to conduct broader sampling for better characterization of mosquito ecology.

Environmental factors, such as temperature and rainfall, are crucial determinants of *Ae. aegypti* female infestation and are related to mosquito development and breeding site availability, respectively [34,35,37]. Rains favor the presence of breeding sites by filling containers stored by household inhabitants or resulting from poor waste disposition [34], as indicated by our analysis. Additionally, the dry season has led people to accumulate water and provide potential breeding sites. In urban areas of La Macarena, local communities prefer to obtain water from boreholes in their backyard and rains, despite having a permanent piped water supply, responding more to historical behavior than the necessity [31,38]. Although we only had enough data to analyze female infestation in urban areas, rural settlements reported the lowest access to piped water and waste collection services, increasing the risk for *Ae. aegypti* colonization as was detected. Health authorities could address these determinants through educational approaches or communication campaigns to refresh the information on potential breeding sites inside households.
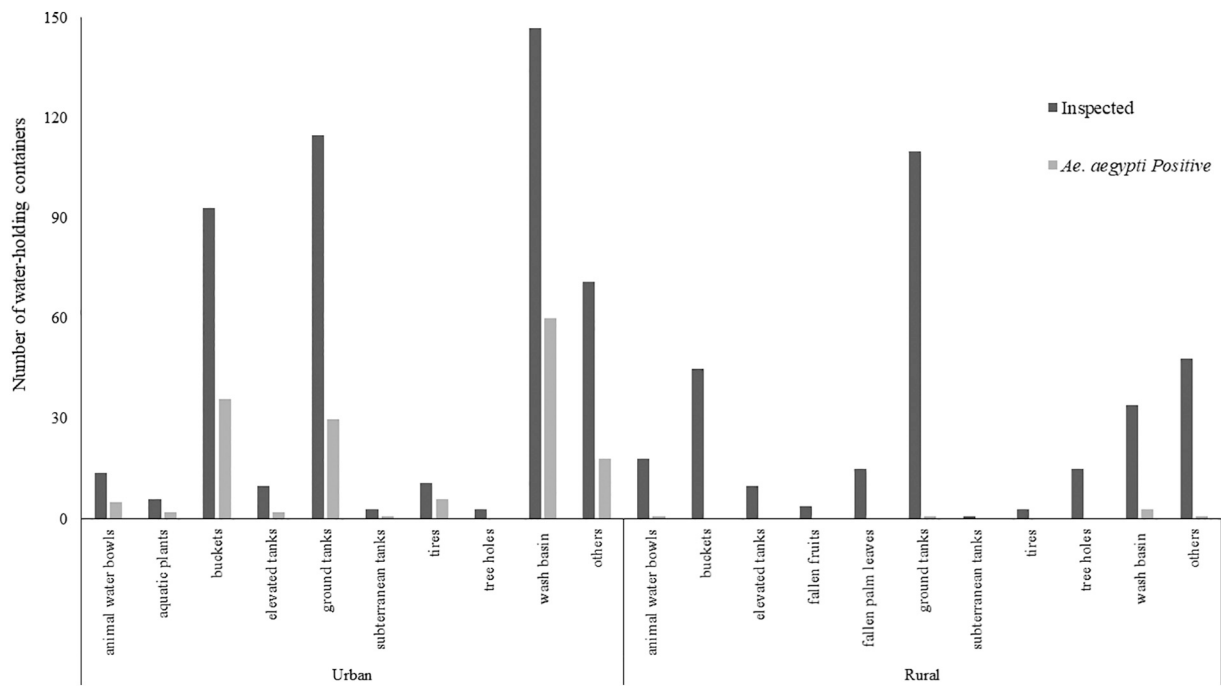
Fig. 3. *Aedes aegypti* breeding places. Inspected water-holding containers and positive records for *Ae. aegypti* presence in urban and rural areas.
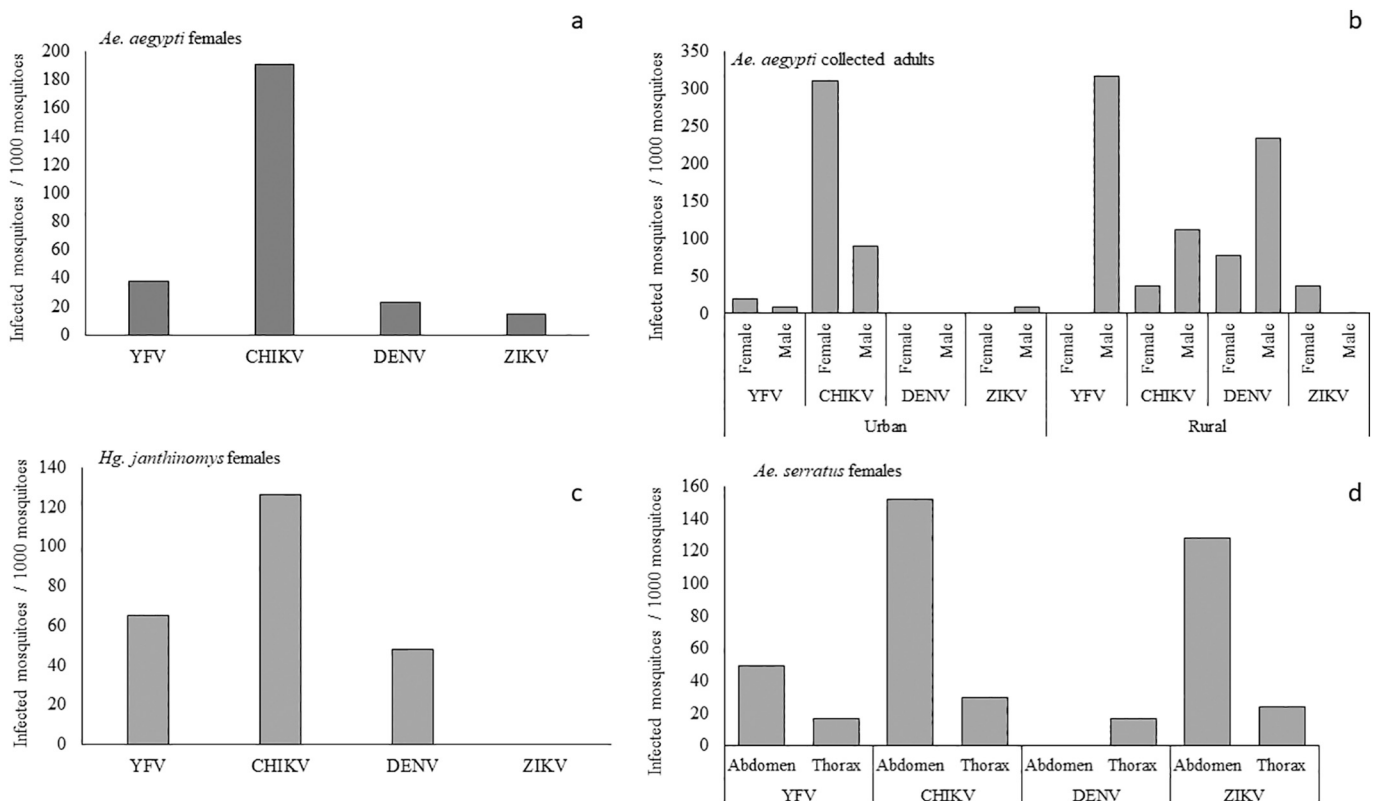

Fig. 4. Infection rate (Firth estimator) from mosquitoes, showing: (a) general infection rates by the four arboviruses observed in the collected and reared females of *Ae. aegypti*, (b) general infection rates by the four arboviruses in *Ae. aegypti* comparing sex and collection area, (c) general infection rates by the four arboviruses in *H. janthinomys* females (d) general infection rates by the four arboviruses found in abdomen and thorax pools of *Ae. serratus* females.

We found a high risk of YFV spillover, as demonstrated by the higher percentage of YFV in *H. janthinomys* (14.3% thorax) than those reported in recent Brazilian outbreaks (6.5–8.7%) [39,40]. We detected YFV circulation in the urban vector *Ae. aegypti*, in contrast to reports from Brazil [3,40] indicating potential rural and urban transmission. In Colombia, YFV vaccination coverage is high (~95%) in the high-risk categorized regions for YFV transmission [38]. Nevertheless, in our vaccination status survey, only 75.9% of participants claimed to be
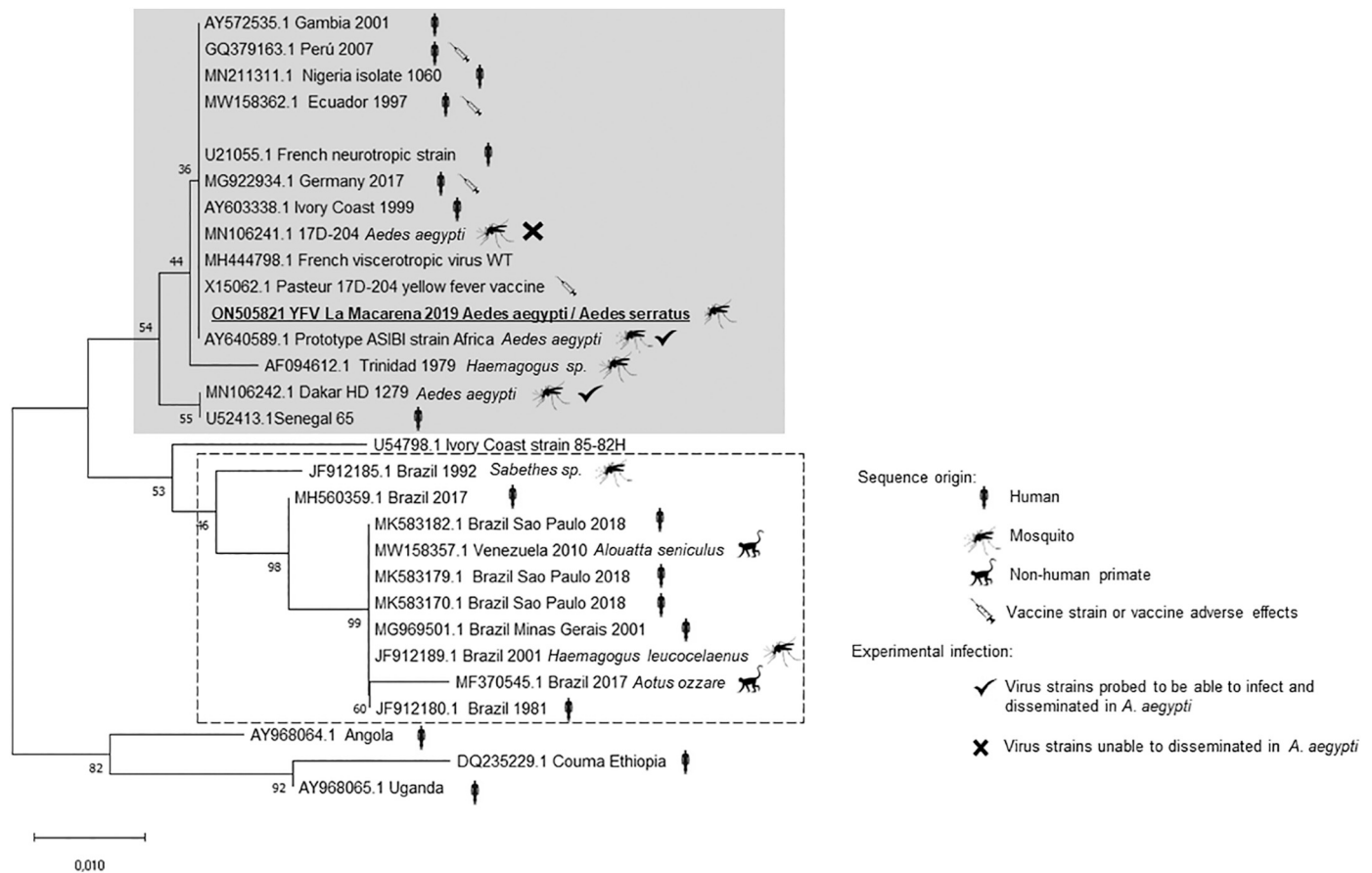
Fig. 5. Phylogram of YFV sequences, using the neighbor-joining method using a fragment of 134 pb of 5UTR and part of capsid gen. The gray area indicates the clade formed for the central African YFV lineages, while the pointing lines indicate the clade formed by the American YFV lineages.

Table 4
Molecular detection of arbovirus (YFV, CHIKV, DENV, and ZIKV) on the different mosquito pools.

Area	Species	Sex/ stage	Positive Pools/ analized	Processed individuals	Collected individuals	Virus found (positive pools /percentage of positivity)*
Urban	*Aedes aegypti*	Females	10/17	50	315	YFV(1/5.8%) CHIK(10/58.8%)
		Males	8/12	111	245	CHIKV (7/58.3%) ZIKV(1/8.3%)
		Larvae	9/13	352	889	YFV (4/30.8%) CHIKV(8/61.5%) ZIKV(1/7.7%) DENV 3 (2/15.4%)
		Reared females	10/16	59	407 pupas	YFV(4/25%) CHIKV(8/50%) ZIKV(1/6.25) DENV 3 (1/6.25)
		Reared males	6/10	90	407 pupas	YFV(2/20%) CHIKV(6/60%)
Rural	*Aedes aegypti*	Females	3/6	11	11	CHIKV (1/16.7%) DENV (2/33%) DENV 2–3(1 16.7%) DENV 2 (1/16.7%) ZIKV(1/16.7%)
		Males	5/6	12	12	YFV (5/83.3%) CHIKV(2/33%) DENV2–3(1/16.7%) DENV 1–3 (2/33%) DENV3 (1/16.7%)
		Larvae	2/2	12	12	YFV (2/100%) CHIKV(2/100%) DENV3(2/100%)
		Pupae	1/1	2	27	YFV (1/100%) CHIKV(1/100%) DENV2(1/100%)
	Haemagogus janthinomys	Females abdomen	6/14	42	46	CHIKV (2/14.3%) DENV (6/42.8%) DENV 3(2/14.3%) DENV1 (4/28.6%)
		Females thorax	5/14	4	46	YFV (2/14.3%) CHIKV (5/35.7%) DENV 1(2/14.3%)
		Females	1/3	3	46	YFV(1/33.3)
		males	1/1	2	2	CHIKV (1/100%) DENV 1,2, 3 (1/100%)
		Larvae	2/2	9	13	DENV3 (1/50%) DENV4 (1/50%)
	Aedes serratus	Females abdomen	19/19	173	421	YFV (8/42.1%) CHIKV(14/73.7%) DENV (18/94.7%) DENV 1 (4/21%) DENV2(4/21%) DENV3(3/15.7%) DENV 1–2 (1/5.2%) DENV1–3 (5/26.3%) DENV 1,3,4 (1/5.2%) ZIKV (10/52.6%)
		Females thorax	7/19	173	421	YFV (3/15.7%) CHIKV(5/26.3%) DENV1(3/15.7%) ZIKV(4/21%)
		Males	6/7	12	18	YFV(4/57.1%) CHIK (5/71.4%) DENV 1(4/57.1%) DENV1,2,3(1/14.3%) DENV2(1/14.3%) ZIKV(4/57.1%)

* Number of positive samples over the number of processed samples per 100.

vaccinated, indicating the potential lowest vaccination coverage in this area, specifically in rural areas. The reported YFV cases in recent years in the country were associated with unvaccinated people from other regions or countries who enter these high transmission risk areas [41].

The YFV molecular sequence obtained was closer to West African sequences (Fig. 5), than to American sequences previously reported [42]. This phenomenon could be associated with the continuous introduction of African YFV into the America [43]. Although, studies examining a longest YFV RNA fragment, need to be conducted to determine the phylogenetic relationship of circulating YFV lineages in mosquitoes. In addition, it was possible to detect other potential arbovirus vectors, such as *Ae. serratus*, in La Macarena, showing YFV, CHIKV, DENV, and ZIKV infections in the thorax of males, indicating the potential dissemination of these viruses, despite contradictory evidence for this species as a potential YFV vector in Brazil [44,45]. Furthermore, we identified CHIKV and DENV in the thorax of *H. janthinomys*. Other studies have reported natural ZIKV infection in *H. leucocelaenus* [46]. However, the virus never reaches the salivary glands [47], as could be the case for our finding, but studies for vectorial incrimination of these mosquitoes need to be conducted.

We found an IR in *Ae. aegypti* similar to that reported in endemic cities in Colombia [48]. Other studies have screened for the presence of viruses in *Ae. aegypti* females and reported the highest infection rates for DENV (0–40.7% of positive pools) and to a lesser extent for CHIKV (0–12.5% of positive pools) [26,49,50]. However, in this study, the most common infection was CHIKV. In 2019, there were 53 DENV cases with no reports of other arboviruses in the La Macarena municipality (Sivigila, http://portalsivigila.ins.gov.co/). The difference between reported human cases and entomo-virological detection could result from asymptomatic, misdiagnosed, and immunological protection from previous exposure.

5. Conclusion

The multidisciplinary approach explored in this research helped us not only to detect an early risk for YFV spillover and rural circulation of other arboviruses, threatening the local community and visitors. But also, was possible to identify entomological, ecological, and social determinants at the regional scale that could be addressed in educational strategies, to decrease the transmission risk. We propose this approach for surveillance in areas of suspected arbovirus transmission for early and effective responses to reduce adverse impacts of these diseases and avoid potential outbreaks.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2022.100438.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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