

Evidence of Co-Circulation of Multiples Arbovirus Transmitted by *Aedes* Sp. Based on Laboratorial Syndromic Surveillance At Health Unit in Slum Area of Federal District, Brazil

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Research Article

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Abstract

Background: Mosquito-borne disease, especially arbovirus transmitted by *Aedes* mosquitoes, must be priority of Brazilian public health policies, mainly with others infectious agents in circulation. Laboratorial syndromic surveillance for fever or/and exanthematic acute syndrome was performed at health unit in slum area of Federal District.

Methods: between June/2019 and March/2020, stopped by COVID-19 pandemic, 131 valid participants were identified and tested by RT-PCR for dengue (by serotype), chikungunya and Zika virus; and by serological IgM for dengue and chikungunya virus, with serologic assay performed when the participant did not present respiratory symptoms (cough or/and coryza).

Results: 3 DENV-1 (2.3%), 4 DENV-2 (3%) and 1 CHIKV (0.7%) was lab-confirmed, showing evidence of hiperendemicity area even with the laboratorial syndromic surveillance not reaching the months of historical peak of dengue in Federal District (April-May). When compared the results of laboratorial syndromic surveillance with traditional epidemiologic surveillance data, is verified significant discrepancy between probable cases (from traditional surveillance) and lab-confirmed cases (from laboratorial syndromic surveillance).

Conclusions: beyond the risks of local population are exposed, the socio-environment profile can be an able potential area to spread arbovirus, according to *Aedes* sp. dynamics and human mobility of the Federal District. Also, traditional surveillance may be misreporting probable cases for dengue infection, and underreporting confirmed cases for other arbovirus in the Federal District.

Background

The emergence and reemergence of neglected infectious diseases (formerly defined tropical diseases) demand continuous monitoring by health agencies responsible for surveillance and intervention, especially in Brazil due to continental size and large proportion of the biomes in constant environmental disturbances, consequently promoting ecological imbalance. Among main emerging infectious diseases, vector-borne and arbovirus transmitted by mosquitoes *Aedes* sp. deserve critical attention from health policies, as observed with dengue infection in the Americas during the two decades of the 21st century: the continent presented the highest magnitude for notifications on the planet, where Brazil contributed with the largest proportion of these numbers in an endemic-epidemic pattern every 3–5 years, according to the prevalence of the serotype [1]. The country has a key role in the amplification and potentiation of mosquito-borne diseases, as quoted by Bedin in 2007 [2], followed by a great example eight years later: the 2015 epidemic of Zika virus transmitted by *Aedes aegypti* that, even its past circulation in other countries, only in Brazil was associated with Congenital Malformation Syndrome [3].

About the main vector *Aedes aegypti* in Brazil - present in all states of the country - we can cited the transmission of the four dengue virus serotypes, since the identification of serotype 1 in 1987, followed by serotype 2 in 1990, serotype 3 in 2000, and serotype 4 in 2010 [4]; the chikungunya virus, introduced in 2013 [5]; and the Zika virus, introduced in 2014 [3]. Furthermore, the species is the urban vector of yellow fever in the country, where repeated outbreaks were observed in the last years associated with sylvatic vectors (*Hemagogus* sp. and *Sabethes* sp.), but in forest fingers near to urban zones [6]. Also relevant, others opportunistic arbovirus (mayaro, o'nyong-nyong, orapouche, etc.) that, according to the vector capacity of *Aedes aegypti* [7], should be attention to its dynamics [8, 9].

This current hyperendemic scenario systematically burdens the Brazilian health system, consequently reflecting on the therapeutic and economic country's capacity, considering yet the complex clinical outcomes associated with arbovirus infection, as neuroinvasives and Guillian-Barré syndromes [10; 11]. In addition, as already mentioned, the Congenital Malformation Syndrome associated with Zika virus, promoting this agent to the STORCH infection group, thus requiring attention in gestational and prenatal preventive programs [12]. The consequences of new arbovirus with similar dynamics of dengue virus promote a more complex transmission landscape, resulting in a wide symptomatological spectrum while decreasing sensitivity and specificity of the clinical-epidemiological diagnosis. The sympatric circulation of different dengue serotypes and other arbovirus also can promote interactions in the pathophysiology and immune response of the human host, resulting in severe outcomes such as shock and hemorrhagic phenomena, until death [13]. Due to the high number of asymptomatic infections [14], as well as false-negative diagnoses according to the lack of accuracy of the methods used routinely [15], outbreaks related to arbovirus can occur without detection of traditional surveillance system in a timely manner, this window facilitating overspread and epidemics [16].

In the present study, laboratorial syndromic surveillance (LSS) was performed for three arbovirus transmitted by mosquitoes *Aedes* sp. at a low-income community (slum) of the Federal District of Brazil, for an estimated period of 1 year at least. This research was realized in the unique health basic unit present at the area, evaluating patients who sought medical support for symptoms associated with acute viral infections, following laboratory confirmation for Dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus (ZIKV), by molecular and serological methods. In Brazil, LSS research for arbovirus transmitted by *Aedes* sp. have already been developed in large cities of the coast where the population is concentrated, such as the Northeast region [10, 14] and Southeastern [17, 18], but was not significantly replicated in the intra-continental region [19, 20]. The study area has already been subject of epidemiological study based on cross-sectional interviews, reporting 28.6% of arbovirus symptoms in the sampled population [21], however without laboratory confirmation. The following topics were analyzed by this research: circulation of DENV, ZIKV and CHIKV in the study area; clinical-epidemiological profile of confirmed cases; the likely local of infection for confirmed cases and possible autochthonous transmissions; comparison between LSS results and traditional epidemiologic surveillance data from study area. Important to highlight, the study was completed in March 2020 due to the COVID-19 pandemic, before the expected period of 1 year of observation (June 2020).

Methods

Study Design

LSS based on defined event for symptomatic infection of arbovirus transmitted by *Aedes* sp. was performed in a health unit of slum area, this health facility being the first destination of local individuals who seek medical support: all individuals who sought the health unit during the morning period (between 8:00 am and 12:00 pm) from Monday to Friday, complaining of febrile and/or exanthematic acute syndrome - the defined event - were invited to participate. The following inclusion criteria were considered for this prospective cohort: being over 18; being residents (sleeping at least four nights a week, for at least three months); and have no contraindication for venipuncture. For exclusion criteria, were considered homeless and not literate (according to sign the Informed Consent Form for research). Verifying the seasonality of the mosquito-borne diseases, for this research a period of 1 year of observation was established, at least (from June 2019 to June 2020).

Following the definition of probable case from the Manual for Adult and Child Diagnosis and Clinical Management for Dengue Fever [22], febrile and/or exanthematic acute syndromes were considered the defined event based on the symptomatology of three arbovirus infection: for DENV, the first manifestations are high fever (39°C to 40°C), associated with cephalgia, myalgia, arthralgia and retroorbital pain, with exanthem presents in 50% of the symptomatic infections, still accompanied by anorexia, nausea, vomiting and diarrhea; for CHIKV, the disease manifestation is similar, with emphasis on the intensity of arthralgias; for ZIKV, fever may present with less intensity or absent, while exanthem occurs more frequently even in the first days of symptomatic infection. Cough and/or coryza, which are not associated with arbovirus infection, was considered for the define event here investigated: therefore, we opted for a wider spectrum than the definition of suspect case (individual showing suggestive signs and symptoms of a group of diseases that share the same symptoms) and probable case (clinically compatible case, without evidence of epidemiological link or/and laboratory confirmation) for DENV, CHIKV and ZIKV, thus reducing possible losses.

The primary identification of patients presenting the defined event at health unit was conducted by the family health teams (FHT), in accordance with the Family Health Strategy [23]. Each FHT is composed by a group of health professionals (medical doctor; nurse; community health agent), and this group are responsible for a limited geographical area from respective region served by the health unit. When a patient seeks the health unit for medical support, the respective FHT makes the first appointment. The health unit where LSS was developed has 12 FHTs, these being oriented to refer for the LSS the patients with suggested symptoms of defined event.

The methodology recommended by Ministry of Health [22] was followed by laboratory confirmation: RT-PCR was performed for DENV (by serotype), CHIKV and ZIKV on acute samples; ELISA-IgM serology was performed for DENV and CHIKV on both acute and convalescent. Considering the serological assay on both moments, two parameters were established according to the positivity: acute infection when seroconversion occurs; and recent infection when serology was positive at the acute moment [14]. Due to the possibility of cross-reaction between dengue and Zika (both flaviviruses), ELISA-IgM was only performed for DENV. Patients who reported cough and/or coryza were not contacted for convalescent collection.

Study Area

LSS was performed at the Health Unit N.º 4 (15 ° 47 '00.89 "S 47 ° 49' 49.96" O) in the Administrative Region of "CidadeEstrutural"(AR: political-administrative unit that configures the territory of Federal District). CidadeEstrutural is less than 10 km from the political center of Brazil, and the respective Executive, Legislative and Judiciary federal powers (Figure 1). Established as a dumping ground in the early 1960s, CidadeEstrutural is one of the least consolidated ARs in the Federal District, consequently with a vulnerable population facing poverty and the corresponding absence of urban planning, infrastructure and environmental sanitation [24]. Currently, as seen in Figure 2, the study area is geographically delimited by Estrutural Highway in the south; by the farm sector in the west; by the industrial AR "SCIA" in the east; and by the Brasilia National Park in the north/northeast. CidadeEstrutural presented approximately 35,730 habitants in 2018, corresponding 1.23% of Federal District population (2,894,953 in same year) [24]. According to epidemiological data of the State Health Secretariat of Federal District, CidadeEstrutural has an important contribution with Federal District numbers: considering probable cases reported by 100,000 inhabitants in 2016, 2017, 2018 and 2019, the indicators of the CidadeEstrutural were, respectively: 1,132; 475; 92.5; 1613, higher than the general incidence at same period, respectively: 583; 141; 79; 1,428.

Interview and Blood sampling

With the patient referred by the FHT, and agreeing to participate, was followed an explanation of the project's objectives, asking him or her to sign the Informed Consent Form. Blood collection was performed using a serum-separating tube (5 mL), and interview was conducted which addressed: demographic and socio-economic characteristics (age, gender, address, environment sanitation conditions of the domicile); day of onset of symptoms and clinical characteristics of the event; daily routine in the last 15 days before the onset of symptoms (school, university, work, travel); possible exposures and epidemiologic link by some close individual with positive diagnosis (by medical doctor) for dengue fever (family, neighbors, co-workers, schoolmates). After 15-21 days from the onset of symptoms, the participants who not reported cough and/or coryza were approached again to a new blood sample collection (5 mL), in addition performing a second part of the questionnaire focusing on the outcome of the symptoms reported at the acute moment (cure or persistence).

Standardized questionnaire was applied using REDCap electronic platform version 7.5.0 (www.project-redcap.org/).

Laboratory Preparation

Tubes with collected blood were kept at room temperature in the health unit laboratory, followed by centrifugation of 1500 x g/10 min, separating 0.5 mL of the supernatant serum in three microtubes (1.5 mL). Properly labeled with the respective patient code, the samples were first kept in the freezer of the health unit laboratory (-20° C), to be transported by thermal boxes to the biorepository of Center for Tropical Medicine, UnB (-80° C), where were kept until molecular and serological analysis.

Viral RNA extraction

Extraction of viral RNA from serum collected at the acute moment was performed using the QIAamp Mini kit, as recommended by the manufacturers (www.qiagen.com/us/resources), from 140 µL of serum resulting 60 µL of eluted.

Reverse transcription and Arboviral RNA detection

RT-PCR was performed using a ZDC multiplex kit from the Institute of Molecular Biology of Paraná from 38 µL of eluted, following the manufacturers' recommendations (www.ibmp.org.br/en-us/). In this protocol, a standardized 96-well microplate is subdivided into four isometric subgroups containing 24 wells, applying the respective PCR mixture according to the primers and probes for the target arbovirus (DENV 1/4; DENV 2/3; CHIKV; ZIKV), with 1 Positive Control and 23 samples to be tested. RT-PCR was performed using QuantStudio 5 (<http://thermofisher.com/>), with the results being analyzed by company's software.

Serological testing for anti-DENV IgM

Both samples collected (acute and convalescent) were tested for anti-DENV using Panbio Dengue IgM Capture ELISA kit, as recommended by the manufacturers (www.globalpointofcare.abbott). The reading was performed on a Kasuaki absorbance reader

(450 nm), using Panbio units for cut-off values, following the criteria: <9 non-reactive; between 9 and 11 inconclusive; > 11 reactive for anti-DENV IgM.

Serological testing for anti-CHIKV IgM

Both samples collected (acute and convalescent) were tested for anti-CHIK using EuroimmunChikungunyaIgM ELISA kit, as recommended by the manufacturers (www.euroimmun.com). The reading was performed on a Kasuaki absorbance reader (450 nm), using relative units (RU/mL) for cut-off values, following the criteria: <0.8 RU/mL negative; between 0.8 and 1.1 RU/mL inconclusive; >1.1 RU/mL positive for anti-CHIKV IgM.

Statistical Analyses

Frequencies of defined event and lab-confirmed cases were organized by epidemiological week (EW) concerning the day of onset of symptoms, according to the period covered by the research. Clinical-epidemiological characteristics were crossed with lab-confirmed cases, by frequency tables. Information obtained from the interviews was used to estimate the likely local of infection. Traditional epidemiologic surveillance data from Cidade Estrutural was arranged by EW and compared with LSS results, using probable cases for DENV (clinically compatible case, without evidence of epidemiological link or/and laboratory confirmation). Statistical difference between probable cases from traditional epidemiology surveillance and LSS results was verified using Wilcoxon matched pairs signed rank test, according to the subject of analysis is paired (by EW), the use of discrete count variables, and respective no-parametric distribution (overdispersion).

Microsoft Excel was used to management data sets, and to design tables and graphs. Stata 14.0 Software was used for statistical analysis. Google Earth was used for satellite images. GPS points from residences were collected using a Garmin Etrex 10 device.

Results

Due to the COVID-19 pandemic and respective growing concern being observed in the first quarter of 2020, the research had to be completed in March. At this time, 9 months of study was performed, between the third week of June 2019 (EW 25 of 2019) and the third week of March 2020 (EW 12 of 2020). The demographic and socio-environmental characteristics of the identified individuals are showed in Table 1.

Table 1
Demographic and socio-environmental characteristics of identified individuals by LSS at Health Unit of Cidade Estrutural, between June 2019 and March 2020.

Variable	Values (N = 131)	
	Lab-confirmed positive (n = 9)	Lab-confirmed negative (n = 122)
Age, mean and median (18–70)	33.3 and 38	32 and 28
Gender (female)	55.5%	62.3%
Ethnicity (no-whites)	100%	89.3%
Years in school, mean and median (0, never attended; 16, university complete)	8.6 and 8	9.2 and 11
Without health insurance	100%	96.7%
Presence of sewage collection at the domicile	66.6%	82%
Presence of piped water supply at the domicile	66.6%	82%
Water rationing had occurred at domicile in the last 3 months	44.4%	40%
Stores water by other methods	44.4%	33,6%
Garbage is collected by service at domicile	66.6%	84,4%

Figure 3 summarizes the patient flow from arrival at the health unit to laboratory confirmation. 157 individuals presenting the defined event were identified by the FHS, of which 134 were valid for the research. 3 valid patients did not have their information saved electronically because operational problems. At the end, 131 defined events were reported. For the second blood collection (convalescent moment), considering that 73 defined events presented cough and/or coryza, 15 refusals and 9 canceled by pandemic, 36 individuals were reached. From the 131 acute samples tested for RT-PCR, 7 were positive for DENV: 3 for serotype 1, and 4 for serotype 2; for ELISA-IgM serology, 3 were positive for DENV (classified as recent infection). Considering the 36 convalescent samples tested for ELISA-IgM, 4 seroconverted to DENV (3 of these corresponding to positive samples on RT-PCR); and 1 seroconverted to CHIKV. The residence of each lab-confirmed case was geo-referenced (Fig. 2).

Figure 4 shows three epidemic curves during study period, these curves adjusted according to acute or convalescent moment and respective laboratory methodology. DENV-2 and dengue seroconversion (DENV-IgM) were identified between EW 25 and 30 of 2019. Otherwise, DENV-1 and CHIKV were identified between EW 4 and 9 of 2020. Between EW 30 of 2019 and EW 4 of 2020 there are no lab-confirmed cases (from 49 defined events). Despite the significant increase of defined events in the lasts EW before the study was interrupted, only one lab-confirmed case was identified in acute samples.

Clinical-epidemiological characteristics of the 131 defined events and respective laboratory confirmation are showed in Table 2. Considering the five most common symptoms associated with acute viral infections (headache, fever, myalgia, arthralgia and retro-orbital pain), practically all were present in lab-confirmed cases, except for 1 case of DENV-1 without arthralgia; and 2 cases of DENV-2, without arthralgia and retro-orbital pain. Cough and coryza were seen in one case of DENV-1 and one case of DENV-2.

Table 2
Clinical-epidemiological characteristics and laboratory diagnosis of 131 defined events identified in Health Unit of Cidade Estrutural:

Laboratory results	TOTAL TESTED N = 131	DENV-1 (n = 3)	DENV-2 (n = 4)	DENV-IgM (n = 1)	CHIK (n = 1)	Negative (n = 122)
Cephalaea	128	3	4	1	1	119
Fever	125	3	4	1	1	116
Myalgia	126	3	4	1	1	117
Arthralgia	103	2	3	1	1	96
Retroorbital pain	102	3	3	1	1	94
Nausea	92	1	3		1	87
Difficulty swallowing	77	2	2		1	72
Shortness of breathe	55		2		1	53
Conjunctivitis	55	2	1	1		51
Diarrhea	44	1	2			41
Exanthem	19	2		1		16
Vomit	35		2			33
Itching	33	1		1		31
Cough	66	1				65
Coryza	62		1			61
Sore throat	62				1	61
Bleeding (nose, mouth, when goes to bathroom)	18	2				16
Oral mucosa lesion	15	1				14

Table 3 presents the main characteristics related to daily routines and possible exposures, for each lab-confirmed case. Observing the individuals represented by samples 39 (DENV-2), 98 (CHIKV) and 106 (DENV-1), there was no other place of exposure besides their residences, thus being able to characterize these cases as domicile transmission. For individual 27 (DENV-IgM), Cidade Estrutural can also be considered a probable site of infection. For individual 37 (DENV-2), the workplace is in the neighboring AR (SCIA, see Fig. 2) but during the night - when the activity of *Aedes aegypti* mosquitoes decrease significantly [25] -, thus also suggesting domicile transmission.

Table 3

Infection characteristics, blood collection dates, daily routines and possible exposures of lab-confirmed cases of arboviruses in Health Unit from Cidade Estrutural:

Samples	Lab-diagnoses	Day of onset of symptom (dd/mm/yy)	Acute blood collection (dd/mm/yy)	Convalescent blood collection (dd/mm/yy)	Daily routine	Travel last 15 days	Exposure last 15 days	Acute Symptoms
9	DENV-2	16/06/19	18/06/19	01/07/19	Unemployed/ not attending school or university	Rural zone of other AR	No	cephalea; fever; myalgia; arthralgia; retroorbital pain; diarrhea
27	DENV-IgM	29/06/19	02/07/19	14/07/19	Work at daytime in study area/ not attending school or university	No	Co-worker with dengue fever	cephalea; fever; myalgia; arthralgia; retroorbital pain; conjunctivitis; itching; exanthem
31	DENV-2	06/07/19	08/07/19	-	Mobile work at daytime in whole FD/ not attending school or university	Rural zone of State of Goias	Co-worker and neighbor with dengue fever	cephalea; fever; myalgia; arthralgia; nausea; shortness of breath; coryza; difficulty swallowing
37	DENV-2	17/07/19	19/07/19	01/08/19	Work at nighttime in study area/ not attending school or university	No	Co-worker and neighbor with dengue fever	cephalea; fever; myalgia; arthralgia; retroorbital pain; nausea; vomit; diarrhea; shortness of breath; conjunctivitis; difficulty swallowing
39	DENV-2	18/07/19	23/07/19	02/08/19	Work at home/ not attending school or university	No	Family member in the same residence with dengue fever	cephalea; fever; myalgia; nausea; retroorbital pain; vomit

Samples	Lab-diagnoses	Day of onset of symptom (dd/mm/yy)	Acute blood collection (dd/mm/yy)	Convalescent blood collection (dd/mm/yy)	Daily routine	Travel last 15 days	Exposure last 15 days	Acute Symptoms
86	DENV-1	21/01/20	23/01/20	-	Work at daytime in other AR/ not attending school or university	No	Neighbor with dengue fever	cephalea; fever; myalgia; arthralgia; retroorbital pain; nausea; cough; conjunctivitis; difficulty swallowing; itching; exanthem
98	CHIK	11/02/20	14/02/20	26/02/20	Unemployed/ not attending school or university	No	No	cephalea; fever; myalgia; arthralgia; retroorbital pain; nausea; shortness of breathe; sore throat; difficulty swallowing
106	DENV-1	24/02/20	27/02/20	10/03/20	Work at daytime in other AR / not attending school or university	No	No	cephalea; fever; myalgia; arthralgia; retroorbital pain; diarrhea; conjunctivitis; difficulty swallowing; oral mucosa lesion; exanthem; bleeding (mouth, while brushing your teeth)
138	DENV-1	18/03/20	20/03/20	02/04/20	Attending school or university daytime in other AR	No	No	cephalea; fever; myalgia; diarrhea; retroorbital pain; bleeding (feces)

FD: Federal District; AR: Administrative Region.

Figure 5 shows three epidemic curves: the data from traditional surveillance (probable cases for DENV) in Federal District from EW 1 of 2019 to EW 25 of 2020; the comparison of LSS results (defined event) with traditional surveillance data in Cidade Estrutural during the same period; and a more detailed comparison (lab-confirmed cases from LSS) between EW 25 of 2019 and EW 12 of 2020, the period of sampling effort of the study. Both data (traditional and LSS) was organized by EW concerning the day of onset of symptoms. Observing traditional surveillance, significant DENV peaks occurred during first semester for both years in Federal District (2019 and 2020), but only 2019 is observed in Cidade Estrutural. For the difference between probable cases from traditional surveillance (105 cases, between EW 25 of 2019 and EW 12 of 2020) and the results of LSS (126 defined events, with 9 lab-confirmed including CHIKV, for the same period), no statistical significance at 95% IC was observed between probable cases and defined event ($p = 0.1257$), only probable cases with lab-confirmed cases ($p < 0.0000$). Probable cases of CHIK and ZIKV were not used due to the low numbers in Federal District during the observed period of research (2019: CHIK: 146 and ZIKV: 59; 2020: CHIK: 1 and ZIKV:0). In addition, confirmed cases from traditional surveillance (laboratory or/and epidemiological link criteria) were not available in public domain data by AR and EW. The following data was obtained from epidemiologic bulletins of State Health Secretariat of Federal District: in 2019, 2,319 confirmed cases for DENV, with 891 showing alarm signs, 84 severe dengue and 62 deaths; 42 for CHIK; 63 for ZIKV; in 2020, 1,292 confirmed cases for DENV with 758 showing alarm signs, 74 severe dengue and 44 deaths; and zero ($n = 0$) for both CHIK and ZIKV.

Discussion

The achievement of LSS in sentinel areas, according to the dynamic of the infectious disease and respective natural history of transmission and infection, configures advanced epidemiological strategy when compared with traditional surveillance [26]. Considering the arboviruses transmitted by mosquitoes *Aedes* sp., effectiveness of true diagnosis can be limited when not supported by laboratory confirmation [14], also limiting the identification of pre-outbreak patterns, and thus losing the opportunity for intervention and control. Not only LSS must feed the health authorities in real-time [27], also entomological surveillance must be incorporated to block the chain of transmission. These different approaches, which naturally complement each other, configure what is known as integrated surveillance [28], when well implemented reduces transmissions and can even reaching eradication of vector-borne diseases [29,30].

The study observed evidence of circulation of two serotypes of dengue, and the chikungunya virus, with high plausibility that, at least, three individuals were infected at their domiciles (positives for DENV-1, DENV-2 and CHIKV). The possible low number of lab-confirmed cases can be justified due to the inclusion of nonspecific symptoms in the defined event, including respiratory symptoms: this option was adopted to maximize sensitivity in the detection of arbovirus infections by laboratory methodologies, for this using a more comprehensive definition than suspected and probable cases criteria from the Ministry of Health [22]. Cidade Estrutural is a hyperendemic area for arboviruses transmitted by *Aedes* sp. mosquitoes, with the respective population exposed to risks of secondary infections, co-infections and clinical outcomes from areas where this "arboviral soup" occurs [13], although cross-immunity and protective effect is observed between flavivirus infection [31, 32, 33]. Also important, this AR can be an able potential area to overspread these arbovirus to others AR, according to the cosmopolite presence of *Aedes aegypti* in all territory of Federal District, and human mobility (including foreign visitors).

The two serotypes of dengue virus were identified temporally distantly, but confirming the epidemiological data of the State Health Secretariat of Federal District: in 2020 from 416 viral isolations, 384 were DENV-1 and 32 were DENV-2; this pattern of serotypes also observed in 2019 (766 viral isolations: 273 DENV-1 and 493 DENV-2); in 2018 (7 viral isolations, all DENV-1); and 2017 (24 viral isolations: 6 DENV-1 and 18 DENV-2); only in 2016 was observed other serotypes (111 viral isolations: 86 DENV-1, 18 DENV-2, 3 DENV-3 and 4 DENV-4); as well as in 2015 (74 viral isolations: 71 DENV-1, 1 DENV-2 and 2 DENV-3). In comparison with other studies based on LSS in health unit, despite the lower absolute numbers of lab-confirmed cases observed here, proportionally the numbers of DENV positives (6,1%) were higher than those observed by Silva et al. 2019 (3.4%) [14], Ferreira et al. 2020 (0.4%) [10], Carvalho et al. 2020 (0.8%) [17] and Vieira et al. 2019 ($n=0$) [20]. Nevertheless, the numbers for the other two arbovirus were higher in these studies: such differences occur, in addition of each methodology and respective limitations and sampled population, due to the period and city that the study was performed, mainly whether near or during outbreaks and epidemics of respective arbovirus, such as Silva et al. 2019 [14] and the 2015-2016 Zika virus epidemic in Salvador.

The lab-confirmed cases presented the five most common symptoms involving clinical-epidemiological characteristics of acute viral infections, confirming the lack of specificity of arbovirus diagnosis when without laboratory support. Cough and coryza were observed, separately, in one case of DENV-1 and one case of DENV-2, respectively: this aspect of acute viral syndromes is used to discriminate airborne virus infections from others (such as arboviruses), especially in areas where health facilities do not have the structure and logistics to confirm a probable case by laboratory support. Also important, the presence of dengue virus in the respiratory tract, even rare, may be possible, conjecturing yet the possibility of aerosol transmission when in close contact if in this condition [34], example of virus "finding" other path for transmission than the observed way, like sexual transmission by Zika virus [35,36]. Observing the confirmed case of DENV-IgM, and considering the possible cross-reaction that may occur between flavivirus in IgM assay as well as exanthema being suggested for acute ZIKV infection, ZIKV transmission can be suggested for this individual. However, other indicators strongly support DENV-2 infection, such as prevalence of this serotype circulation along with this seroconversion (between EW 25 and 29 of 2019), and the low incidence of ZIKV in Federal District at period (besides CHIKV and ZIKV can be underreported, see paragraph below).

Observing the difference between probable cases of Federal District and Cidade Estrutural (both values from traditional surveillance data) is suggested that Cidade Estrutural did not contribute with Federal District DENV numbers during 2020, the opposite of 2019. Also, COVID-19 pandemic must be considered a potential source of bias for probable case notifications during 2020. The comparison between traditional surveillance (probable cases) and LSS results (defined event and lab-confirmed cases) observed similarity between defined event and probable cases during the study period, this can be justified due to the health unit is the unique in the low-income community, since both variables are supposedly from the same source. However, considering: some limitations in communication between LSS and the FHTs; the LSS performed only during morning period (8:00 am- 12:00 pm); a more comprehensive symptomatology of defined event than probable case; the exclusion criteria for individuals under the age of 18 for LSS; and others possible sources for notification beyond health unit, all these factors can explain observed differences in some EWs for both variables. About the contrasting between probable cases and lab-confirmed cases, it suggests different acute viral infections that is first classified with probable case of dengue infection, highlighting the importance of case investigation and respective confirmation by laboratory or/and epidemiological link [37]. In the view of the limitations of clinical-epidemiologic criteria, as well as the gold pattern of laboratory criteria for infectious diseases diagnosis, and the continual decline of financial support of Brazilian National Health System [38], it can be suggested that majority true-positive cases of infectious diseases in acute phase is not reached by the traditional surveillance, including CHIKV and ZIKV (confirmed cases for both in traditional surveillance in 2020: zero). The worst scenario of this can be observed by the COVID-19 pandemic in Brazil and the mutual circulation of arbovirus transmitted by *Aedes* sp. [39], also the posteriorly endemic circulation of SARs-CoV-2 in Brazilian population.

Some limitations marked the development of the LSS: first, the communication with different FHT, due to the own limitation of health professional staff, produced heterogeneity in the identification of defined events during the study period. Second, the possibility of not maintaining the LSS during the daily operation of the health unit (8:00 am-6:00pm), additionally the lack of second blood collection for serological confirmation in patients with respiratory symptoms, contributes to the reduction of sampling effort for laboratory diagnosis. Third, already mentioned, the COVID-19 pandemic did not allow the research to finish the planned one year of observation period: the pandemic also interrupted the laboratory processing of biological samples, since resources (mainly viral RNA extractor) and workforce were redirected to efforts against the new coronavirus. Some questions remain with the results here observed when concerning the lab-confirmed cases of DENV-2 and DENV-1 are distant at time (respectively, July 2019 and January 2020), and no lab-confirmed cases was observed between these two serotypes clusters: are these transmissions sustained at local level of study area during low density of *Aedes* sp. mosquitoes, according to the respective seasonality of arbovirus ("transmission islands"); or yet, occurs some kind of ecological substitution of DENV serotypes during this seasonality? Nevertheless, to respond these questions, active surveillance must be performed (also focusing asymptomatic infections), as well as entomological surveys.

Conclusion

Despite all limitations, the LSS managed to identify autochthonous transmission at domicile level, for two different serotypes of DENV, and CHIKV, in the study area. When compared these results with traditional surveillance data is observed significant discrepancy, suggesting that other acute viral syndromes may be classified as probable cases of dengue infection, while true-positives diagnosis are not reaching for the epidemiologic surveillance database. Also, considering the significant number of

negative laboratory results for the three arbovirus here investigated (even with possible false negatives), a large number of acute syndromes caused by other virus can be speculated, especially airborne virus (defined events complaining of cough: 66; coryza: 62; both: 53) in attention to those ones that seasonally circulated in the Federal District before the COVID-19 pandemic (influenza virus; adenovirus; rhinovirus; old coronavirus; etc.), highlighting the importance of continuous LSS for infectious diseases. These biological materials are available for future laboratorial analysis (despite have not samples collected by swab, only blood samples). Also important to the COVID-19 pandemic context, as the others coronavirus outbreaks (SARS in 2002; MERS in 2013) and 2015 Zika virus pandemic, the attention to sylvatic virus and other infectious agents that circulate in natural areas and can affect human populations, according to environmental disturbance and respective spillover phenomenon: like seen Figure 2, the advance of the Cidade Estrutural over the National Park without an organization plan for landing use. In order to mitigate the negative impact of anthropogenic actions on the environment, multidisciplinary approaches need to be incorporated in health agendas, such as the use of biodiversity and eco-epidemiological indicators that measure the impact of environmental disturbances and emergence of infectious diseases [40]. Integrated surveillance on respective fronts (traditional, syndromic, laboratory, genomic, entomological, eco-epidemiological), in addition of hybrid systems and new data science methodologies [41], are crucial instruments to prevent the emergence of new infectious agents, as well as the reemergence of old ones, in this brave new world.

List Of Abbreviations:

STORCH: syphilis, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex

LSS: laboratory syndromic surveillance

DENV: dengue virus

ZIKV: Zika virus

CHIKV: chikungunya virus

FHT: Family Health Team

AR: Administrative Region of Federal District, Brazil

FD: Federal District

EW: epidemiologic week

Declarations

Ethics approval and consent to participate

Valid participants were informed about the project's objectives and respective proceedings, as well as the risks and benefits involved. Those who agreed to participate voluntarily signed an Informed Consent Form. The research complied the resolution 466/2012 of the National Health Council and the principles of the Declaration of Helsinki, and was approved by the Ethics Committees in Research involving human beings from University of Brasília (CAAE: 61551116.3.3001.5558); and from State Health Secretariat of the Federal District (CAAE: 61551116.3.0000.5553).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared there are no competing interests in the development of the research.

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Authors' contributions

PRM, MABJ, MPG, RH and WNA conceptualized the study; PRM, MABJ and MPG performed investigation and field collection; PRM performed data curation; PRM, LAGN, TSCQ, TFN, APSC performed laboratorial methodology; PRM, LAGN, TSCQ, TFN, DCCA and RH performed laboratorial analysis; PRM interpreted the results, wrote the original draft of manuscript and designed the visualization; PRM, TSCQ, TFN, RH, GASR and WNA reviewed and edited the final manuscript. All authors read and approved the final manuscript.

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Figures



Figure 1

Satellite image of Federal District focusing Plano Piloto and adjacent area: (A) Cidade Estrutural; (B) political center of Federal District, where is localized the Executive, Legislative and Judiciary chiefs office of Brazil. The inserted map of Brazil indicates the location of Federal District. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

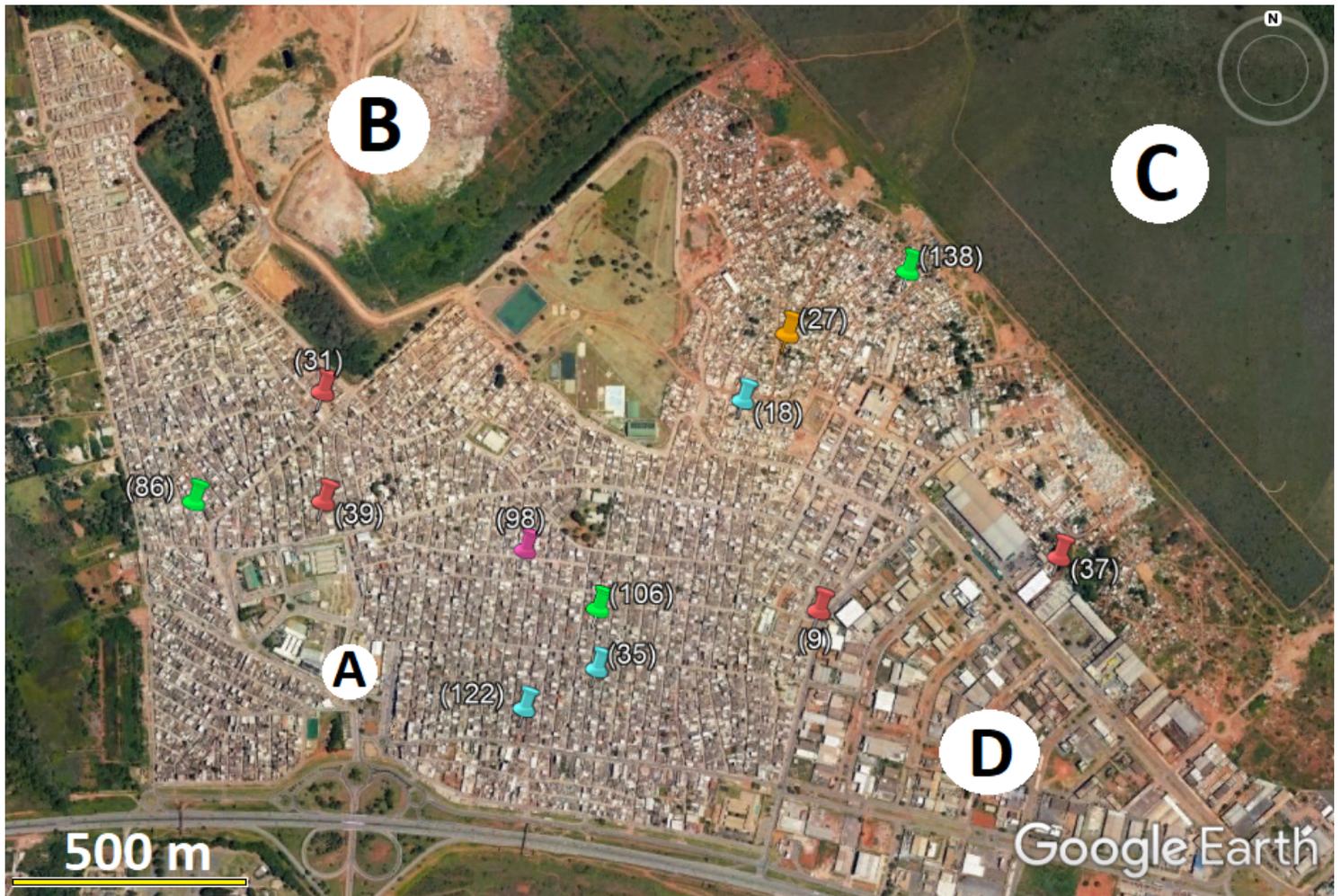


Figure 2

Satellite image of CidadeEstrutural, and domicile of each laboratory positives cases for arbovirus. (A): Health Unit; (B): deactivated dumping ground; (C): National Park of Brasília; (D): industrial AR (SCIA). For each laboratory positive case (domicile pointed), the following colors represent the infection diagnoses: Green=DENV-1; Red=DENV-2; Purple=CHIK; Orange=DENV-IgM acute infection (seroconversion); Blue=DENV-IgM recent infection Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

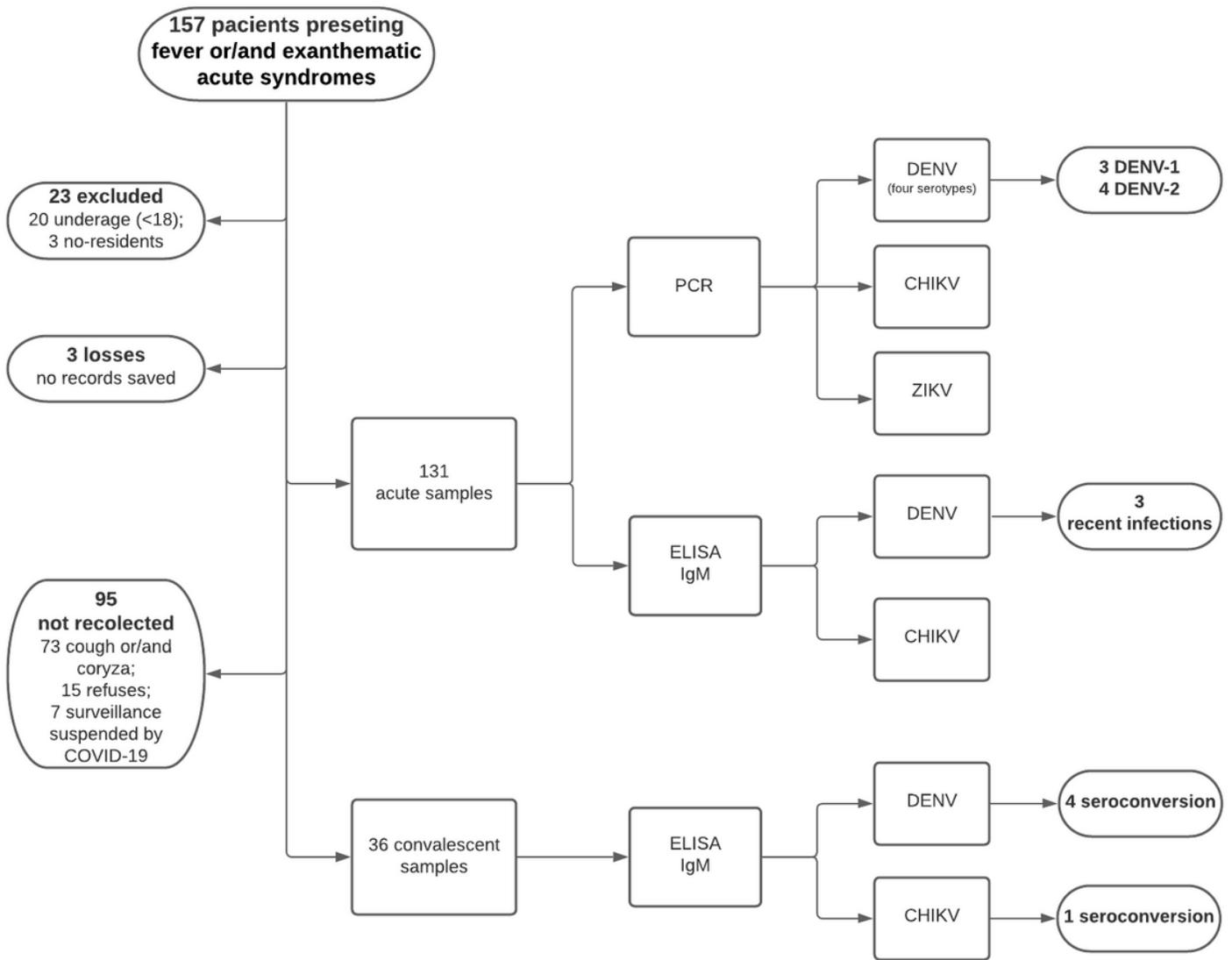


Figure 3

Scheme chart of patient flux of laboratorial syndromic surveillance, Health Unit from CidadeEstrutural.

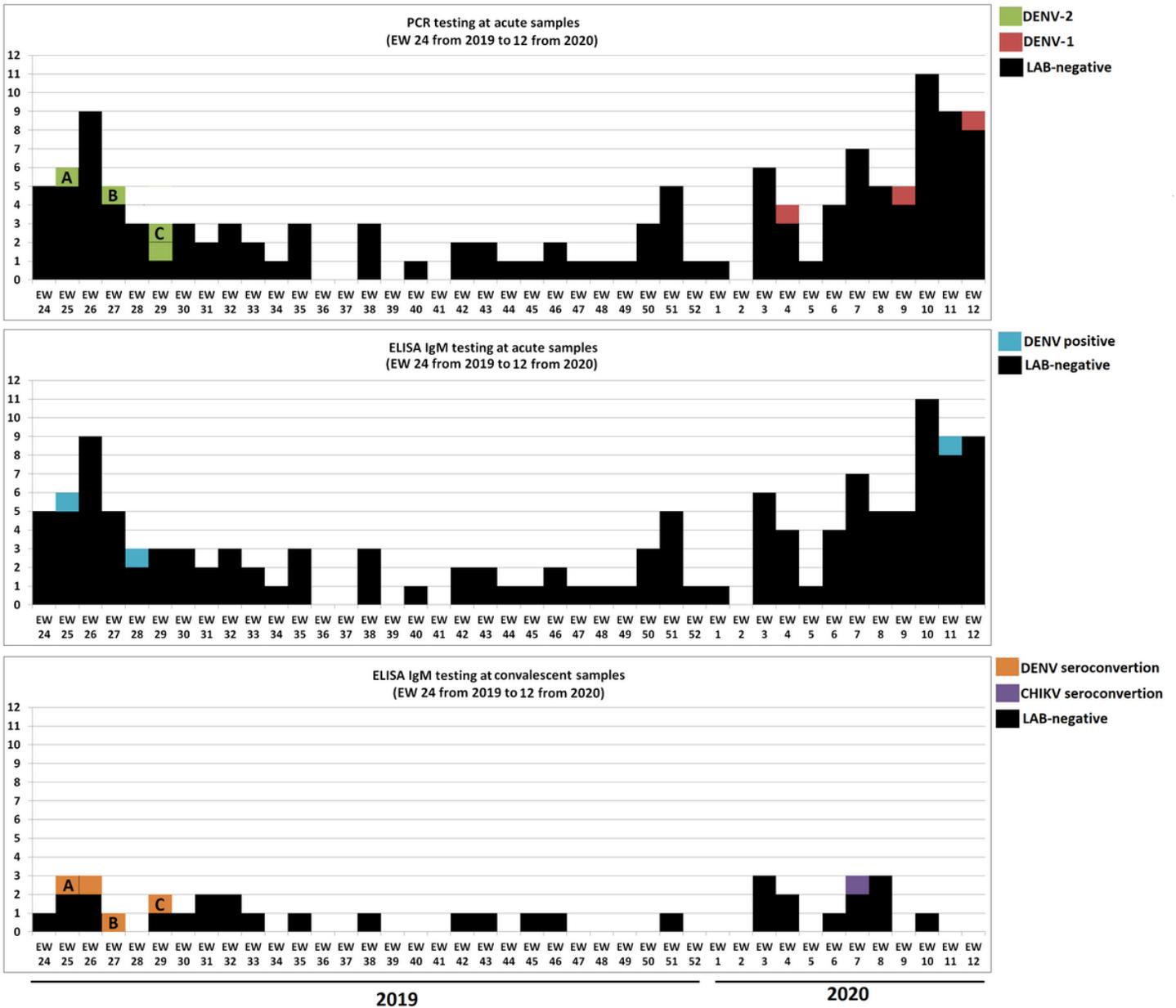


Figure 4

Epidemic curves of the laboratory confirmed cases by date of onset of symptoms, Health Unit from CidadeEstrutural, between EW 24, 2019 and EW 12, 2020, for: PCR testing at acute samples (131); ELISA-IgM testing at acute samples (131); ELISA-IgM testing at convalescent samples (36). The letters on positive cases of DENV-1 (for PCR) and DENV-IgM seroconversion represents the same individual/sample: A (in EW 25), B (EW 27) and C (EW 29).

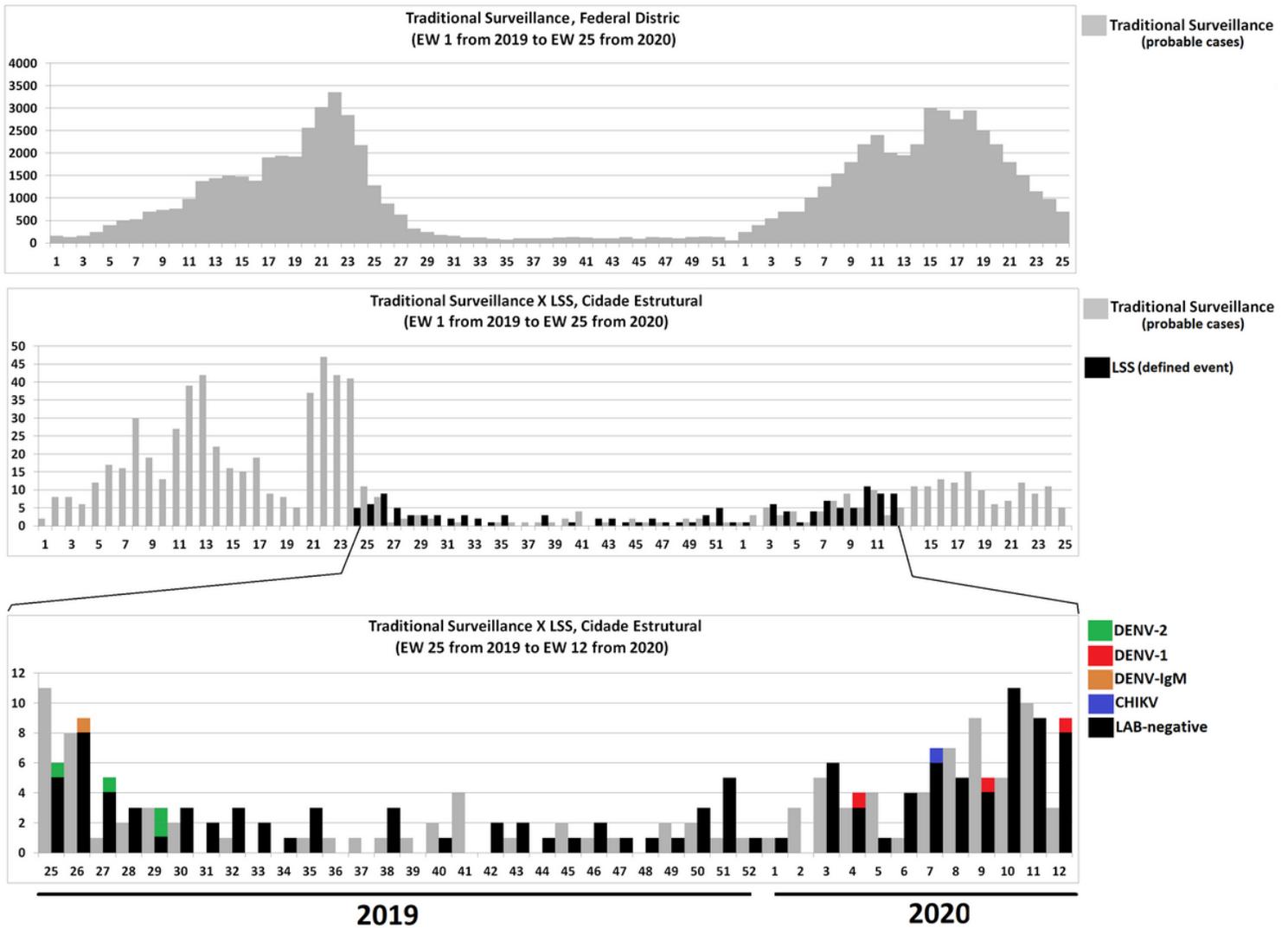


Figure 5

Epidemic curves by date of onset of symptoms for: traditional surveillance (probable cases) for Federal District between EW 1 from 2019 to EW 25 from 2020; traditional surveillance (probable cases) and LSS results (defined event) for CidadeEstrutural between EW 1 from 2019 to EW 25 from 2020; traditional surveillance (probable cases) and LSS results (lab-confirmed cases) between EW 1 from 2019 to EW 25 from 2020.

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