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A comprehensive analysis and immunobiology of autoimmune neurological syndromes during the Zika virus outbreak in Cúcuta, Colombia

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ABSTRACT

We have focused on the epidemiology and immunobiology of Zika virus (ZIKV) infection and factors associated with the development of Guillain-Barré syndrome (GBS) and other neurological syndromes in Cúcuta, the capital of North Santander department, Colombia. Data of patients with ZIKV disease reported to the national population-based surveillance system were used to calculate the basic reproduction number (R_0) and the attack rates (ARs) as well as to develop epidemiological maps. Patients with neurological syndromes were contacted and their diagnoses were confirmed. A case-control study in which 29 patients with GBS associated with ZIKV compared with 74-matched control patients with ZIKV infection alone was undertaken. Antibodies against arboviruses and other infections that may trigger GBS were evaluated. The estimated value of R_0 ranged between 2.68 (95% CI 2.54–2.67) to 4.57 (95% CI 4.18–5.01). The sex-specific ARs were 1306 per 100,000 females, and 552 per 100,000 males. A non-linear interaction between age and gender on the ARs was observed. The incidence of GBS in Cúcuta increased 4.41 times secondary to ZIKV infection. The lag time between ZIKV infection and neurological symptoms was 7 days (interquartile range 2–14.5). Patients with GBS appeared to represent a lower socioeconomic status and were living near to environmentally contaminated areas. All GBS patients were positive for IgG antibodies against both ZIKV and Dengue virus, and 69% were positive for Chikungunya virus. Noteworthy, GBS was associated with a previous infection with *M. pneumoniae* (OR: 3.95; 95% CI 1.44–13.01; $p = 0.006$). No differences in antibody levels against *C. jejuni*, Epstein-Barr virus and cytomegalovirus were observed. High rates of cranial nerves involvement and dysautonomia were present in 82% and 75.9%, respectively. Intensive care unit (ICU) admission was necessary in 69% of the GBS patients. Most of the patients disclosed a high disability condition (Hughes grade 4). Dysautonomia was the main risk factor of poor GBS prognosis (i.e., ICU admission and disability). Thirteen patients were diagnosed with other neurological syndromes different to GBS (6 with transverse myelitis, 3 with encephalitis, 3 with peripheral facial palsy and one with thoraco-lumbosacral myelopathy). Our data confirm an

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increased transmission of ZIKV in Cúcuta, and provide support to the view that severe neurological syndromes are related to ZIKV disease. The complex ways by which previous infections and socioeconomic status interact to increase the risk of GBS in people infected by ZIKV should be further investigated.

1. Introduction

Infection by Zika virus (ZIKV), an arbovirus of the *Flaviviridae* family, is transmitted by the female *Aedes* mosquito genus. The response to infection normally varies between completely asymptomatic individuals to those with mild and self-limiting disease [1]. In such individuals, the typical symptoms include rash, fever, arthralgia, and conjunctivitis. However, in Latin America and in the South Pacific, from where it is believed the spread originated, there have been increasing reports of neurological complications attributable to ZIKV. The sudden increase in microcephaly and Guillain-Barré syndrome (GBS) [2–4] prompted the World Health Organization (WHO) to declare a "public health emergency of international concern" [4]. According to the Colombian National System of Public Health Surveillance (SIVIGILA for "Sistema Nacional de Vigilancia en Salud Pública"), between epidemiological week (EW) 32 of 2015 to week 28 of 2016, the date on which the closure of the epidemic phase of the disease was declared, 99,721 cases of ZIKV disease were registered, of which 8826 (8.9%) were defined serologically and confirmed by reverse transcription polymerase chain reaction (RT-PCR) [5].

Cúcuta, the capital of North Santander department of Colombia, is located in the northeast of the country at the border between Colombia and Venezuela and is the most affected Colombian city for arboviruses [6]. It also has one of the largest proportions of registered cases of neurological syndromes related to ZIKV disease [5]. This finding prompted us to evaluate the factors associated with the development of these neurological syndromes in Cúcuta.

2. Methods

2.1. Setting

Cúcuta, located at latitude 07° 53′ 00″ N and longitude 72° 30′ 19″ W, is 320 m above sea level, and has a territorial area of 1176 km^2. In 2016, the Administrative National Department of Statistics (DANE for "Departamento Administrativo Nacional de Estadística"), estimated the total population as 656,380 habitants (sex ratio: 93%), distributed in 54,500 homes, in 600 neighborhoods with a population density of 592 people km^2; the urban area accounts for 96.62% of the population. The climate is warm and characterized by temperatures ranging between 59 and 104 °F (15–40 °C); in the months of April to June and September to November. There is an average annual rainfall of 655 mm. The annual average relative humidity is between 70 and 75% [7].

2.2. Public Health Surveillance system

In Colombia, the National Institute of Health (INS for "Instituto Nacional de Salud") maintains the SIVIGILA for notifiable clinical cases that include Dengue (DENV), Chikungunya (CHIKV), ZIKV, and birth defects, among others. The information gathered by the centers of health care is compiled and transmitted to the SIVIGILA, which updates the results weekly [8]. In addition, all cases of neurological syndromes (GBS, ascending polyneuropathy, encephalitis, peripheral facial paralysis, among other similar neurological conditions), with suspicion of ZIKV disease are also provided to the SIVIGILA [9].

2.3. Patients and controls

In this restrospective case-control study, those patients with previous probable infection with ZIKV associated with a neurological syndrome registered in the SIVIGILA were considered as cases. The control group was defined as patients with previous probable infection with ZIKV, registered in the SIVIGILA that did not develop GBS or another neurological syndrome. These people were matched to GBS patients by age (±5 years), gender, neighborhood and date of ZIKV symptoms (±2 EW).

In order to evaluate the neurological conditions and to obtain samples from patients, a multidisciplinary team that included neurologists, immunologists, physicians and research assistants conducted 4 site visits to Cúcuta. Patients were diagnosed with GBS according to the Asbury [10,11] and Brighton criteria [12]. Transverse myelitis and encephalitis were diagnosed according to the transverse myelitis consortium working group [13], and the international encephalitis consortium [14], respectively. Data were collected in an electronic and secure database as reported elsewhere [15].

Medical examination and blood samples were done and withdrawal respectively, within a median of 108 days (IQR: 97.75–134) after the onset of the Zika symptoms and within a median of 100 days (IQR: 85–116) after the onset of the GBS.

2.4. Data collection

Data for the studies reported herein covered the time period from June 29, 2015 to July 30, 2016. In fact, retrospectively, a sentinel case was registered on June 29, 2015 [16]. The data monitoring system included all 65 health care centers within the city. Non-resident's cases in the city were excluded from analysis.

The date of GBS onset was defined as the first subjective experience of the symptom(s) and/or sign(s) as described in the classification criteria. Additional criteria included cranial nerve involvement, dysautonomia, intensive care unit (ICU) admission, respiratory failure, mechanical ventilation requirement, onset and type of treatment. Disability was assessed according to the Hughes' scale [17].

Other characteristics evaluated in this study included: level of education, socioeconomic status, smoking habits, coffee intake and exposure to toxic agents. The educational level was recorded as the number of years of education. This variable was divided into three groups (0 years, 1–9 years and more than 9 years of education) based on the Colombian General Law of Education [18]. The socioeconomic status was assigned based on criteria outlined by the Colombian legislature [19]. The smoking habits were assessed as no previous history of smoking, patients who consumed 1 to 5, 6 to 15, and more than 15 packs/year, or as an ex-smoker. Coffee intake was explored as a yes/no question, measured in cups intake per day (i.e., less than 1 and 2 to 4). Exposure to toxic agents such as organic

solvents, hair dye, pesticides and fuels were also recorded.

Perceived morbidity including the history of previous infections such as hepatitis, pneumonia, DENV, CHIKV and ZIKV was registered as well as records of previous vaccination and co-morbidities. The data recorded on each patient included previous symptoms related to ZIKV and other infections, such as fever, rash, arthralgia, conjunctivitis, diarrhea, and respiratory symptoms. Data on controls consisting of demographic characteristics, perceived morbidity and symptoms related to ZIKV were also recorded.

2.5. Electrophysiological studies

Electrophysiological studies performed were subjected to the same analysis by an expert neurophysiologist (E.O.). The electrophysiological assessment was made by standard techniques. Motor conduction velocity studies, such as compound muscle action potentials and amplitude was evaluated in the median, ulnar, peroneal and tibial nerves; sensory nerve action potential and conduction velocity was measured in median, sural and ulnar nerves. We also registered F wave and H reflex, in order to determine proximal nerve segments. Exploratory electromyography study was performed for both the upper and lower limbs [20,21].

2.6. Laboratory studies

In order to confirm previous ZIKV infection, ZIKV IgG and IgM antibodies were measured utilizing standardized enzyme-linked-immunosorbent assays (ELISA). In addition, considering that the city of Cúcuta is an endemic region for DENV and CHIKV, antibodies against these viruses were also quantified. Since GBS can also be triggered by some common infections [22,23], antibodies against *Mycoplasma pneumoniae*, *Campylobacter jejuni*, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were likewise quantified. Sera from the patients with GBS were analyzed for anti-ganglioside antibodies.

2.6.1. ZIKV, DENV and CHIKV diagnosis

Detection of IgG and IgM in serum samples against ZIKV, DENV and CHIKV were quantified using an ELISA from Euroimmun (Luebeck, Germany), Vircell (Granada, Spain) and Abcam (Cambridge, United Kingdom) respectively. ELISAs were performed as specified by the manufacturer. In addition, detection of IgG against ZIKV, CHIKV and each of the 4 serotypes of DENV was performed using an indirect immunofluorescence assay (Euroimmun, Luebeck, Germany) on serum samples from each patient. Negative and positive controls provided by the manufacturer were analyzed in parallel.

2.6.2. Other infectious diseases diagnosis

Serological testing for IgM and IgG specific antibodies against CMV, *M. Pneumoniae* and EBV were performed by ELISA (Vircell, Granada, Spain). IgG antibodies against *C. jejuni* were also quantified by using an indirect semi quantitative ELISA from Euroimmun (Luebeck, Germany). ELISAs were performed as specified by the manufacturer and included both positive and negative controls assayed in parallel.

2.6.3. Anti-ganglioside antibodies

Antibodies against gangliosides were measured in the sera from the 29 patients diagnosed with GBS. IgG and IgM antibodies against GA1, GM1, GM2, GD1a, GD1b, and GQ1b were measured by ELISA kits commercially obtained from Bühlman Laboratories (Shoenenbuch, Switzerland). Whereas GM1 specific antibodies were measured by an independent ELISA, the other gangliosides were measured using a GanglioCombi ELISA (Bühlman Laboratories, Schoenenbuch, Switzerland). ELISAs were performed as specified by the manufacturer. Moreover, in order to increase specificity and to extend the number of gangliosides evaluated, IgG and IgM antibodies against GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b were also assessed by an immunoblot assay (Euroimmun, Luebeck, Germany). This technique consisted of test strips coated with parallel lines of purified antigens, which were incubated with dilutions of the patients' samples followed by the addition of an enzyme-labelled anti-human IgG or IgM antibody and the addition of the appropriate substrate. The assay was performed according to the manufacturer's instructions. The reactivity was scored positive based on the reactivity of the positive control on a strip run in parallel and provided by the kit.

2.7. Statistical analysis

For descriptive results, categorical variables are presented as proportions and continuous variables by the median and interquartile range (IQR). The basic reproduction number, R_0, defined as the average number of secondary cases generated by a typical infectious individual in a fully susceptible population, was estimated assuming an exponential growth rate model and a gamma distribution with a mean value of 22 and 2 standard deviations (SD) for the generation time distribution as described by Chowell et al. [24]. The initial number of cases was one, following the available SIVIGILA's first report. Poisson regression of incidence was used to fit the model as implemented by Obadia et al. [25]. Sensibility analysis was performed to assess the effect of different parameters on the generation time distribution of the R_0 estimate. The ranges of the mean and SDs were 18–27 and 0.8–5 days, respectively, and were considered by biological plausibility.

The overall and age/sex-specific attack rates (ARs) were calculated by using population census data from DANE. A generalized additive model (GAM) was used to assess differences in ARs between sexes and age groups, and particularly to model the nonlinear pattern associated with age. The smoothing function used to adjust the GAM model was a thin plate regression spline based on automatic selection of the effective degrees of freedom, as implemented in GAM function of mgcv R package by Wood et al. [26]. Surveillance data were analyzed using R version 3.2.0 [27].

The mixed-cluster methodology based on multivariate descriptive methods such as multiple correspondence analysis was performed to summarize sets of related variables with strong associations and common clinical context [28]. A bivariate analysis

Table 1
Major characteristics of reported cases of Zika virus disease in Cúcuta, Colombia, 2015–2016.[a]

Characteristic	N (%) or median (IQR)
Female	4382 (71.2)
Age, years	28 (21–38)
Time between onset of symptoms and date of notification, days	3 (1–6)
Type of case	
Probable	5462 (89,3)
Laboratory-confirmed	655 (10,7)
Hospitalized	191 (3.1)
Pregnant	1936 (44.2)
Age, years	25 (21–30)
Congenital abnormalities	6 (0,1)
Neurological syndrome	66 (1,1)
Age, years	35 (26–47)
Male	34 (51,6)
Time between reported viral syndrome and onset of neurological symptoms (days)	0 (0–3.8)

[a] According to the Colombian National System of Public Health Surveillance (SIVIGILA for “Sistema Nacional de Vigilancia en Salud Pública”).

was performed in order to evaluate whether some of the main outcomes in GBS (ICU admission, respiratory failure, and Hughes' scale), were modified by ZIKV infection as follows: the chi square and Fisher's exact tests were performed to establish differences among categorical variables. Kruskal-Wallis test was performed for assessing possible differences in continuous variables on categorical outcomes. The incidence of GBS after the outbreak of ZIKV in Cúcuta was calculated dividing the total estimated number of GBS cases by the total population of Cúcuta, multiplied by 100.000.

2.8. Geographic distribution of neurological cases

Surveillance case data (2015–2016; officially reported by the INS through the SIVIGILA) were used to develop the maps and estimate the cumulative incidence rates using reference population data on ZIKV infections (cases/100,000 population). ArcGIS, a geographic information system software, was used for the development of epidemiological maps. To identify critical points of contamination including sites where discharge of waste water occurs, we used information provided by the company entrusted to cleaning the city and those responsible for collection and disposal of suction waste of the city. We took into account the farmers market as a point of contamination, due to residual waters used in the market place.

2.9. Ethics statement

This study was performed in compliance with Act 008430/1993 of the Ministry of Health of the Republic of Colombia, which classified it as minimal-risk research. All of the patients voluntarily

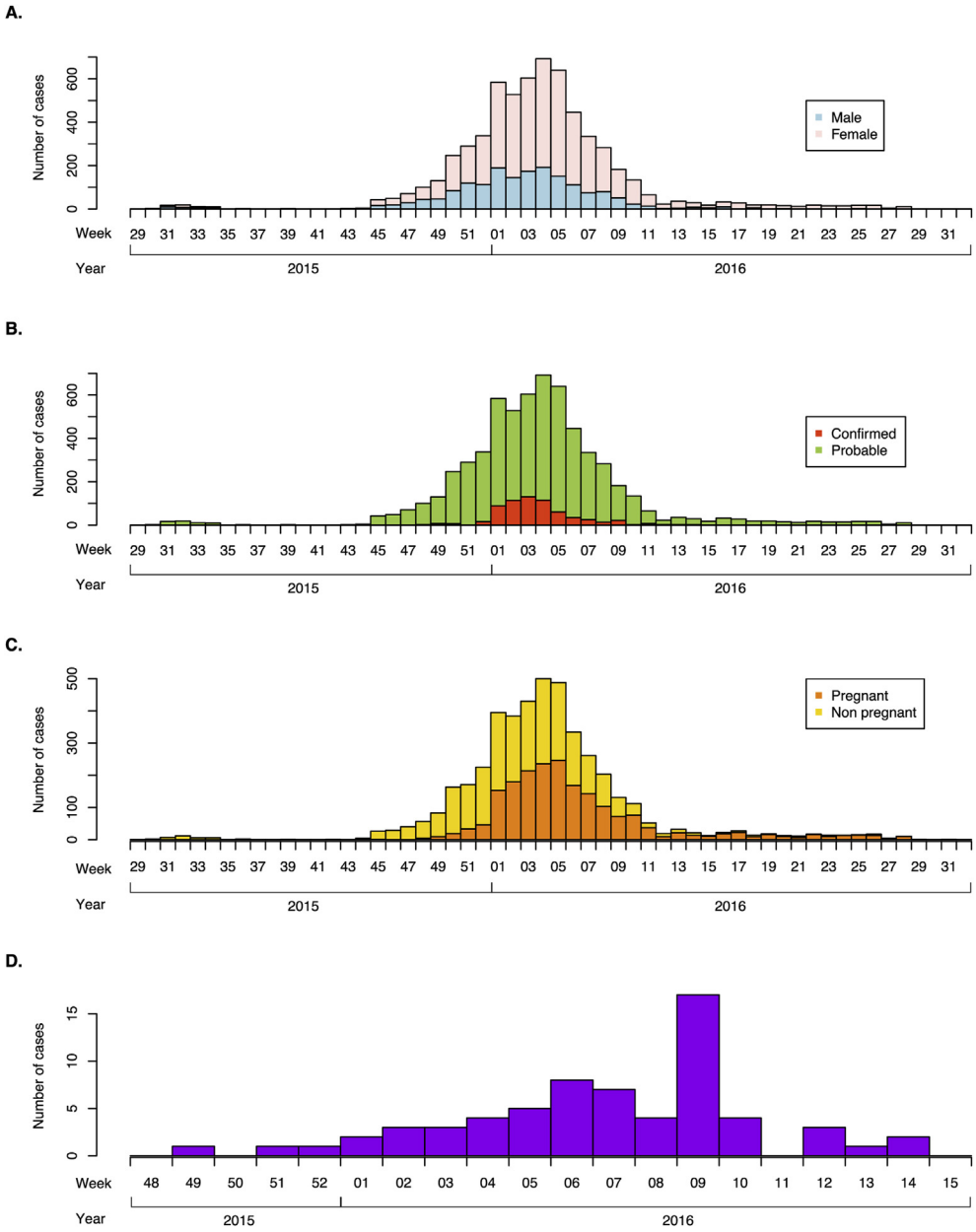


Fig. 1. Epidemiology and transmissibility of Zika virus (ZIKV) disease in Cúcuta, Colombia, 2015–2016. Weekly epidemic curves for different groups of ZIKV disease in Cúcuta. All individuals reported to the Colombian National System of Public Health Surveillance (SIVIGILA for "Sistema Nacional de Vigilancia en Salud Pública") with symptoms of ZIKV disease, with and without laboratory confirmation were included. A. Distribution by gender. B. Distribution according to confirmed and probable cases. C. Distribution by pregnancy status. D. Neurological syndromes reported.

accepted to participate in the study by reading and signing the informed consent document. The institutional review board of the Universidad del Rosario approved the study design.

3. Results

3.1. Geoepidemiology of ZIKV disease

By July 30, 2016, there were 6117 cases of ZIKV disease reported by the 65 health centers in Cúcuta, of which 655 (10.7%) were confirmed by RT-PCR assay (Table 1). The median age was 28 years. Age distribution was similar between male and female cases ($p = 0.59$). The overall ratio of ZIKV disease was 2.5 women per affected male (Fig. 1A). The epidemic presented a unimodal pattern of distribution (Fig. 1B). The outbreak reached a maximum number of 720 cases during week 01 of 2016 with decreasing numbers subsequently reaching a stable number of reported cases by week 11 of 2016. The median number of cases per week was 12 (IQR: 4–90), and 72% of the cases were reported during 2016.

Among the risk groups prioritized by the INS, they were 56/224 (25%) children < 1 year of age, 518/1936 (27%) pregnant women and 37/207 (18%) people ≥ 65 years who were laboratory-confirmed cases. There were 1936 pregnant women reported with ZIKV disease, representing 44.3% of the female population recorded in the study period, with a median of 10.5 cases per week (Fig. 1C). Pregnant women were younger than non-pregnant women (25.7 ± 6.0 vs. 33.5 ± 19.36 years, $p < 0.001$). There were 6 cases of congenital anomalies associated with ZIKV disease reported to the SIVIGILA.

Neurological syndromes registered (N = 66) represented 1.1% of the ZIKV disease cases (Fig. 1 D). The first one occurred in the EW 49 during 2015, and the last in the EW 14 during 2016, with a maximum number of cases in week 09 of 2016; 51.5% of these neurological cases were male (Table 1). Sensibility analysis disclosed an estimated value of R_0 ranging between 2.68 (95% CI 2.54–2.67) to 4.57 (95% CI 4.18–5.01). The effect of the assumed mean and SD for the gamma generation time distribution indicated a positive proportional relation between the mean of the distribution and the R_0 estimates, whereas the relation between the SD and R_0 estimates was the opposite (Fig. 2).

The sex-specific ARs were 1306 per 100,000 females and 552 per 100,000 males. GAM analysis revealed a complex relationship between ARs associated with age and gender. AR differences between gender were most important within the age range between 15 and 39 years, where female AR were significantly higher than those of males of the same age ($p < 0.0001$). Another important fact was the high AR for children under 1 year of age. This group had the highest AR among men, and a similar trend was observed in women. However, women of age between 25 and 29 years had an AR similar to those of girls under one year of age ($p = 0.31$) (Fig. 3).

The geo-reference was possible in 41 of the patients with neurological syndromes. As shown in Fig. 4, neurological syndromes were geo-referenced close to farmer's market and discharges of tailings water. The neighborhoods with higher cases report of GBS were Chapinero with 3 cases, Aeropuerto, Alfonso López and Motilones two cases each. Otherwise, the neighborhoods Ospina Pérez and Sevilla had the higher incidence of other neurological syndromes, with 2 cases each (Fig. 4 and Table 2).

3.2. Guillain-Barré syndrome associated with ZIKV disease

The patients with neurological syndromes were invited to participate in the present study as outlined in Fig. 5 and the medical records were collected and revised. From the 66 patients, 15 were

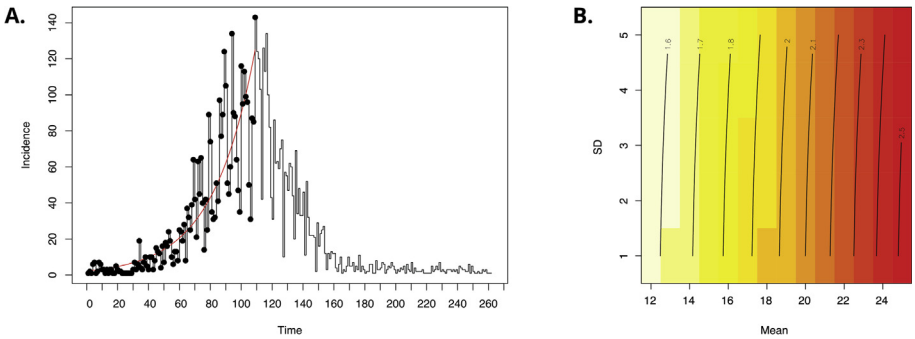


Fig. 2. A. Daily epidemic curve used to estimate R_0. Red line corresponds to exponential growth model adjusted to the early phase of the outbreak. B. Heat map of sensibility analysis for generation time distribution parameters. Colors and level lines correspond to R_0 estimated values under specific mean and standard deviation values for the gamma distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

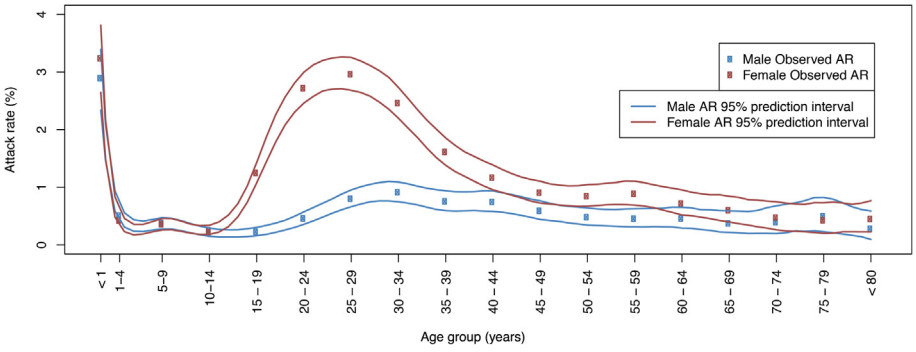
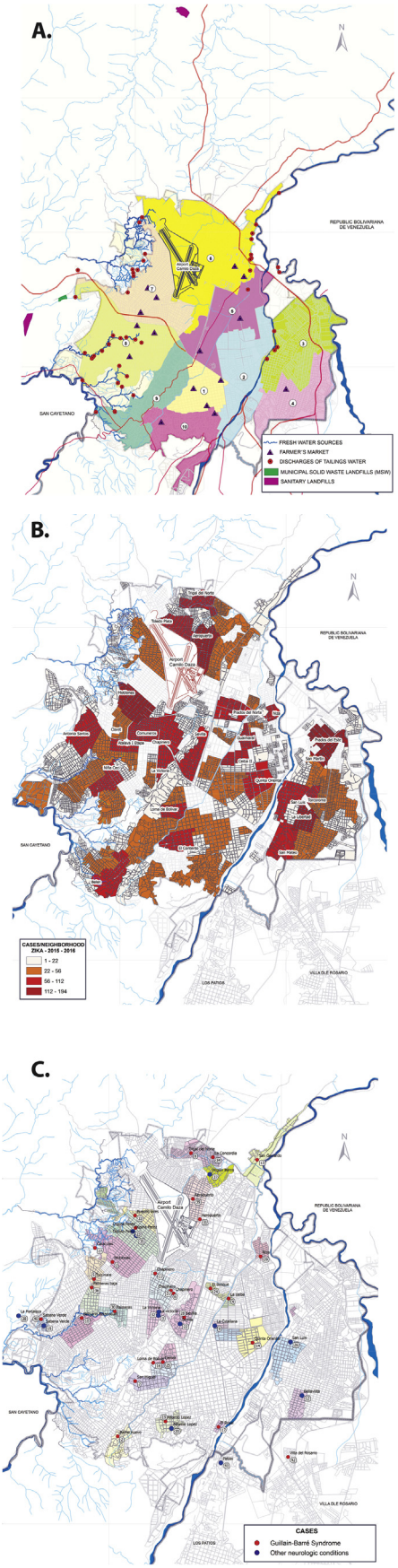


Fig. 3. Age and gender specific Zika virus disease attack rates (ARs).



withdrawn from the study due to several reasons, which included the finding that 3 were not living in Cúcuta, 9 had no evidence of neurological syndrome and 3 had incomplete medical records. Three additional patients that were not included in the previous surveillance but showed up during the site visits and met the criteria were also included bringing the total number of neurological cases to 42. A total of 29 out of the 42 patients were diagnosed with GBS and the remaining 13 were classified as patients with other neurological disorders (Fig. 5). Twenty-one (72.4%) patients fulfilled the Brighton level 1 criteria, and 8 (27.6%) patients fulfilled level 2 criteria. The control group was composed of 74 patients with ZIKV infection and no history of neurological disease.

Taken into account that the reported incidence of GBS is one or two cases per 100,000 people every year [22,23], the incidence of developing GBS in Cúcuta increased 4.41 times secondary to ZIKV infection. The clinical characteristics of the patients in the GBS group are shown in Table 3. The most salient demographic difference for increased incidence of GBS was low socioeconomic status (Table 4). Other risk factors included a mixed occupation history, a student occupation and manual labor occupation. No significant differences were found between cases and controls regarding perceived morbidity, vaccinations and co-morbidities. No significant relationship was evident between cigarette smoking and coffee consumption and the development of GBS (data not shown). All patients had a history of infection by ZIKV with a median of 7 days (IQR: 2–14.5) before the onset of neurological symptoms.

Patients with dysautonomia were grouped into three groups and the data analyzed using the mixed cluster analysis in order to evaluate the influence of this clinical complication (Fig. 6). In the first group, 20% of the patients reported problems with both urinary retention and ileus. In group 2, all the patients showed lability of blood pressure, followed by urinary retention (23.1%) and ileus (7.7%). In group 3, labile blood pressure and ileus were the most frequent dysautonomia, reported by 100% of the patients, followed by urinary retention in 83.3%, diaphoresis in 66.7% and arrhythmias in 50% of patients. Within the GBS group, 69% of the patients were admitted to the ICU (Table 3). Most patients received treatment within 7 days after the onset of symptoms (55.1%). Treatment consisted on intravenous immunoglobulins (IVIg) in 21 patients (72.4%), one patient was treated with the combination of IVIg and plasmapheresis (3.4%), one patient received IV glucocorticoids, and the remaining 6 patients received not particular treatment. The median hospital stay was 23 days (IQR: 9.5–36) and none of the patients died.

Trunk weakness (OR: 4.8; 95% CI 1.28–36.02; $p = 0.0144$) and dysautonomia (OR: 6; 95% CI 1.47–55.57; $p = 0.008$) were associated with ICU admission. Patients in dysautonomia group 3 presented a higher proportion of ICU admission than patients in dysautonomia group 1. (OR: 7.2; 95% CI 0.83–424.17; $p = 0.008$). Nevertheless, this association should be taken with prudence due to the small sample size. Labile blood pressure was the most common variable in this study (OR: 4.8; 95% CI 1.28–36.02; $p = 0.0144$). On the other hand, acute inflammatory demyelinating polyneuropathy (AIDP) subphenotype was negatively associated with ICU admission (OR: 0.08; 95% CI 0.02–1.01; $p = 0.026$).

The second evaluated outcome was respiratory failure, which was highly associated with trunk weakness (OR: 4; 95% CI 1.06–30.27; $p = 0.0271$) and dysautonomia. The group 3 patients presented a higher proportion of respiratory failure than patients in

Fig. 4. Geographic distribution of neurological cases A. Critical contamination points. B. Incidence rate of ZIKV disease (cases/100, 000 population) in Cúcuta, showed by communes. C. Geographic distribution of patients with Guillain-Barré Syndrome and other neurological conditions.

Table 2
Zika virus incidence rate (cases/100,000 population) per neighborhood, Cúcuta, Norte de Santander, Colombia.[a]

Neighborhood	Cases 2015–2016	%	Population 2016	Incidence (cases/100.000 pop)
Cúcuta	6078	100	656,414	925,9
Aeropuerto	135	2,77	11,725	1151,4
Aguas Calientes	50	3,59	7860	636,1
Alfonso Lopez	79	4,89	4893	1614,6
Alto Pamplonita	28	5,43	7691	364,1
Aniversario I	40	6,09	2276	1757,5
Aniversario II	44	6,82	4699	936,4
Antonia Santos	109	8,61	24,008	454,0
Atalaya i etapa	75	9,84	11,290	664,3
Atalaya iii etapa	7	9,96	2408	290,7
Barrio Blanco	18	10,37	1827	985,2
Barrio Nuevo	16	10,63	6118	261,5
Belen	84	12,02	10,663	787,8
Belisario Betancourt	47	12,94	6019	780,9
Bellavista	47	13,71	7708	609,8
Bocono	32	14,24	1229	2603,7
Bogota	7	14,35	1776	394,1
Brisas del Aeropuerto	13	14,85	5063	256,8
Brisas del Pamplonita	3	15,34	2483	120,8
Buenos Aires	30	15,96	4149	723,0
Camilo Daza	35	16,69	1372	2550,8
Camilo Torres	17	16,97	2686	633,0
Caña Fistolo	4	17,05	1552	257,7
Caobos	28	17,86	2832	988,8
Carlos Pizarro	15	18,10	3460	433,5
Carlos Ramirez Paris	20	18,43	5214	383,6
Carora	45	19,17	4986	902,5
Cecilia Castro	7	19,29	1644	425,9
Ceiba ii	78	20,57	1842	4233,9
Cerro la Cruz	4	20,64	3477	115,0
Cerro Norte	7	20,75	3799	184,3
Chapinero	91	22,35	11,678	779,3
Colinas de la Victoria	1	25,06	245	408,6
Colsag	39	25,85	3351	1163,8
Comuneros	108	27,63	13,772	784,2
Condado de Castilla	1	27,65	313	319,4
Cucuta 75	29	29,31	3478	833,7
Divina Pastora	28	30,61	4796	583,8
Doña Nidia	54	31,56	7108	759,7
El Contento	94	34,67	12,653	742,9
El Desierto	7	34,79	2265	309,0
El llano	39	35,44	4283	910,6
El Progreso	16	36,14	1634	979,2
El Rosal	24	36,56	1179	2035,2
El salado	35	37,14	4981	702,7
Gaitan	33	37,90	4220	782,0
Galan	14	38,13	1473	950,6
Gratamira	7	39,71	1466	477,4
Guaimaral	82	41,05	9256	885,9
Gualanday	5	41,14	687	727,7
Juana Rangel	4	41,42	1124	355,8
La Cabrera	52	42,32	4421	1176,1
La Castellana	4	42,70	2581	155,0
La Ceiba	29	43,18	8964	323,5
La Concordia	32	43,70	279	11,473,3
La Florida	6	43,97	825	727,3
La Hermita	34	44,53	4681	726,4
La Libertad	194	47,95	7958	2437,8
La Mar	7	48,16	1434	488,1
La Merced	3	48,21	2131	140,8
La Playa	36	48,80	4022	895,0
La Primavera	10	48,97	1528	654,4
La Rinconada	2	49,02	344	581,2
La Riviera	15	49,26	1448	1035,9
La Union	29	49,74	3007	964,4
La Victoria	73	50,94	9106	801,7
Latino	13	52,29	2158	602,5
Libertadores	15	52,54	1533	978,2
Lleras Restrepo	30	53,15	3202	936,9
Loma de Bolivar	74	54,36	9540	775,7
Los Acacios	22	54,73	3868	568,7
Los Almendros	26	55,15	3427	758,7
Los Alpes	15	55,40	2996	500,6

(*continued on next page*)

Table 2 (*continued*)

Neighborhood	Cases 2015–2016	%	Population 2016	Incidence (cases/100.000 pop)
Los Pinos	14	56,55	2830	494,8
Manolo Lemus	7	56,70	13,745	50,9
Motilones	173	60,37	13,872	1247,1
Niña Ceci	77	61,65	10,797	713,1
Niza	74	62,87	4550	1626,4
Nuevo Escobal	22	63,40	2283	963,8
Ospina Perez	76	65,31	16,544	459,4
Panamericano	40	66,77	4363	916,8
Policarpa	12	68,56	1813	661,7
Popular	20	68,89	1624	1231,3
Prados Club	10	69,50	1225	816,6
Prados del Este	131	71,66	3199	4095,0
Prados del Norte	84	73,04	7080	1186,4
Prados i	1	73,06	1657	60,4
Puente barco	6	73,83	1418	423,3
Quinta Bosh	36	74,42	5697	631,9
Quinta Oriental	75	75,65	7455	1006,1
Rafael Nuñez	4	75,72	519	770,2
Rudesindo Soto	17	76,07	3211	529,4
San Gerardo	7	76,67	1065	657,0
San Luis	112	79,36	5915	1893,6
San Martin	123	81,38	4798	2563,5
San Mateo	69	82,52	6037	1143,0
San Miguel	55	83,42	10,061	546,7
San Rafael	52	84,28	6933	750,0
Santa Ana	48	85,07	3490	1375,3
Santa Lucia	3	85,39	2525	118,8
Santa Teresita	14	85,63	6391	219,0
Santander	39	86,27	4630	842,3
Santo Domingo	43	86,97	3572	1203,7
Sevilla	72	88,18	12,635	569,8
Toledo Plata	83	90,59	5078	1634,5
Torcoroma	76	91,84	3181	2389,0
Torcoroma i	6	91,94	3181	188,6
Torcoroma ii, sector i, ii, iii	30	92,44	827	3627,6
Trigal del Norte	96	95,71	481	19,963,5
Tucunare	39	96,35	7192	542,3
Valle Esther	19	97,31	3832	495,8
Virgilio Barco	34	99,05	2641	1287,6

[a] According to the Colombian National System of Public Health Surveillance (SIVIGILA for "Sistema Nacional de Vigilancia en Salud Pública"), epidemiological week 30, 2016, and the Administrative National Department of Statistics (DANE for "Departamento Administrativo Nacional de Estadística"), 2016.

dysautonomia group 1 (OR: 4.37; 95% CI 0.86–71.38; p = 0.0389). Similar to the association noted above for dysautonomia, the group 3 patients showed the highest association with respiratory failure, with arrhythmias being the most important clinical indicator (OR: 3.79; 95% CI 0.99–29.17; p = 0.033).

During the functional evaluation of each patient using Hughes' scale (post hospital discharge), 52% of the patients were at grade 4 (Table 3). Certain variables as being men (OR: 4.4; 95% CI 1.26–28.137; p = 0.0159), cranial nerve involvement (OR: 3.9; 95% CI 0.86–43.70; p = 0.038), dysautonomia (OR: 3.9; 95% CI 0.86–43.70; p = 0.038), mainly arrhythmia (OR: 8; 95% CI 1.76–90.36; p = 0.0033) and requirement of invasive mechanical ventilation (OR: 4.95; 95% CI 1.29–38.36; p = 0.013), were associated with Hughes grade 4 outcome.

Cúcuta is an endemic region for ZIKV, DENV and CHIKV, since they share the same *Aedes* genus vector. Therefore, IgG and IgM antibodies for the three viruses were quantified using two different techniques, due to the high degree of cross-reactivity of antibodies against the flaviviruses previously reported [29]. As mentioned, the serological study was performed within a median of 108 days after the onset of the Zika symptoms and within a median of 100 days after the onset of the GBS.

All patients from the GBS group were positive for anti-DENV and anti-ZIKV IgG antibodies, while only 69% of the patients were CHIKV positive. Since IgM antibodies against ZIKV and DENV were only positive in 3.45% and 17.2%, respectively, it is highly unlikely that the antibody response noted were due to recent ZIKV and DENV infection. No patients were positive for anti-CHIKV IgM antibodies. Beyond the serological results obtained by ELISA, IgG antibodies against ZIKV, CHIKV and the four serotypes of DENV were analyzed by an immunofluorescence assay. There was 99% agreement between the ELISA based seropositivity and the data obtained using the immunofluorescence technique for ZIKV and CHIKV. While DENV-1, -2 and -3 IgG antibodies were found in sera from 96.5% of the patients, DENV-4 antibodies were noted in 100% of the sera analyzed.

Concerning other previous infections, sera from all the patients with GBS reported IgG antibodies against CMV and EBV. For *C. jejuni* only IgG antibodies were measured, and were found to be present in 24.14% of GBS patients and in 16.22% of controls, with no significant difference. While sera from none of the patients had detectable levels of IgM antibodies against *M. pneumoniae*, it was interesting to note that sera from 82.76% of the patients showed IgG antibodies against *M. pneumoniae* as compared with 54.05% of the control subjects (OR: 3.95; 95% CI 1.44–13.01; p = 0.006). Perception of pneumonia did not correlate with a previous *M. pneumoniae* infection.

One GBS patient was positive for IgG antibodies against GM1 measured in an individual ELISA and one patient was positive for anti-GQ1b IgG antibodies by GanglioCombi ELISA. Regarding immunoblot results, no positivity for IgG antibodies was found for any ganglioside. However, sera from six patients were positive for

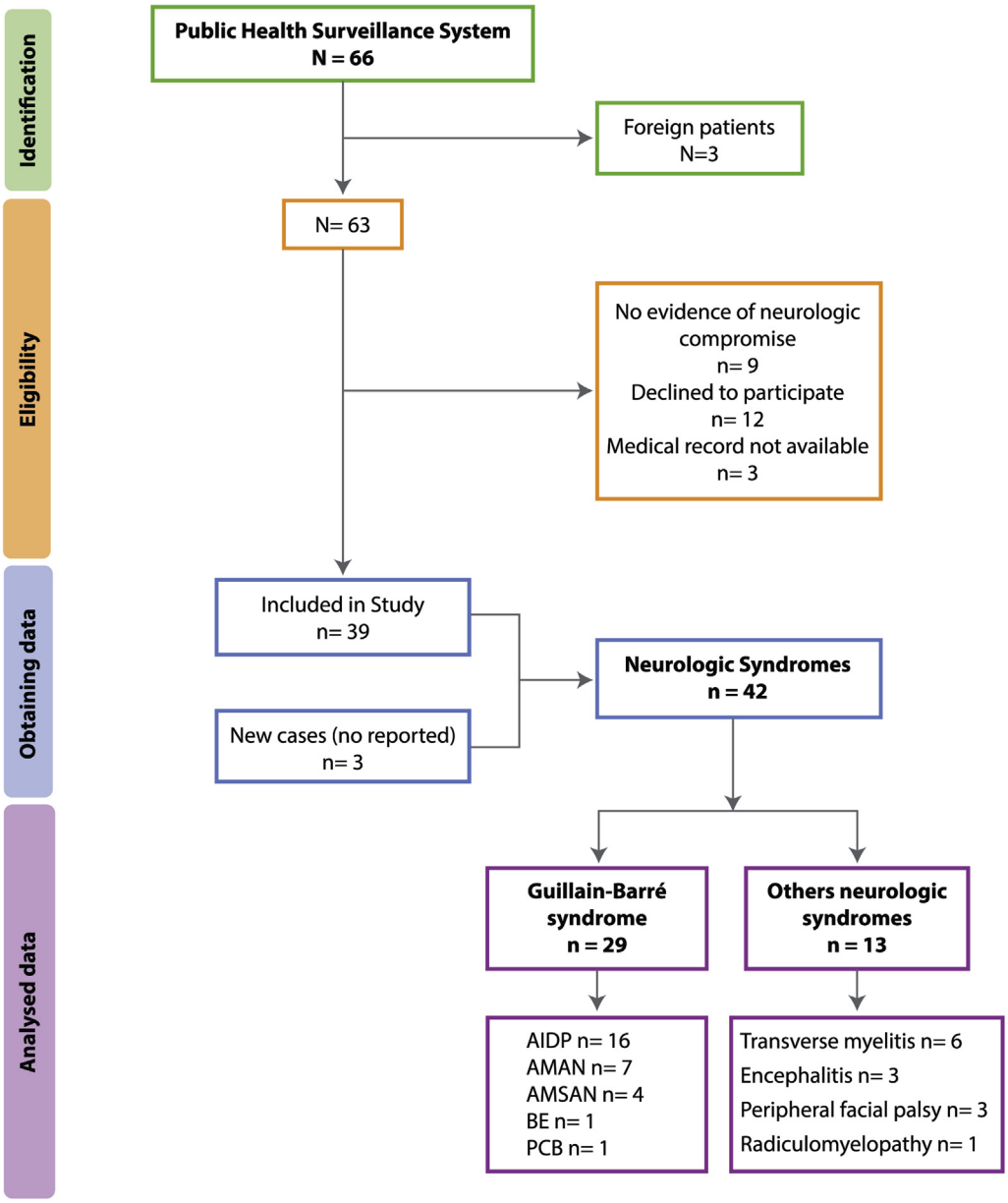


Fig. 5. Flow diagram of patient recruitment. PRISMA IPD. Acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN), Bickerstaff's encephalitis (BE), pharyngeal-cervical-brachial (PCB) variant.

IgM antibodies against some of the gangliosides measured. Sera from two patients had IgM antibodies against GM1 and GM2, two for GM1 and GT1b, one for GQ1b and GT1b and only one for GM1. Antiganglioside antibodies were found in sera from 71.43% of patients disclosing AIDP and in 14.28% of patients with axonal forms (AMSAN). There was no available information for one patient.

3.3. Other neurological syndromes associated with ZIKV disease

Thirteen patients were diagnosed with other neurological syndromes different to GBS. Infectious profile of these 13 patients is shown in Table 5. Within this group, 6 presented with transverse myelitis and 3 with encephalitis. Three additional patients presented with peripheral facial palsy and one with thoracolumbosacral myelopathy (Fig. 5). Clinical characteristics of patients with transverse myelitis are shown in Table 6.

Patients with encephalitis had a median age of 33 years (IQR: 27.5–35), and 2 were male. All the 3 patients presented with altered mental status and fever. The lag time between ZIKV and neurological disease was 8 days (IQR: 5–8). In 2, generalized or partial seizures and meningeal signs were observed. Lumbar puncture, performed in 2 patients, disclosed pleocytosis in one. No specific findings were observed by diagnostic imaging. Two patients were admitted to the ICU.

Patients with facial palsy had a median age of 26 years (IQR: 24.5–33), all patients were women and were diagnosed with ZIKV infection with a median of 12 days (IQR: 6–49.5) before the onset of neurological symptoms. All patients reportedly reached a peak of clinical severity prior to 72 h of diagnosis. They presented decrease or loss of movement in all ipsilateral facial muscles, associated with ipsilateral sensory disturbances. Patients recovered within 2–3 weeks after onset of symptoms, without any neurological sequelae.

Table 3
Clinical characteristics of patients with GBS associated with ZIKV infection.

Variable	N = 29 (%)
Age (IQR)	42 (34–49)
Men	15 (52)
Lag between ZIKV and neurological disease, days. Median (IQR)	7 (2–14.5)
Lag between symptoms and diagnosis, days. Median (IQR)	2 (1–6)
Paresthesia	26 (90)
Symmetric weakness	28 (96.5)
Lower limb weakness	27 (93)
Upper limb weakness	28 (96.5)
Trunk weakness	19 (65.5)
Hypo-areflexia lower limbs	27 (93)
Hypo-areflexia upper limbs	26 (90)
Cranial nerve involvement	24 (82.7)
VII cranial nerve only	8/24 (33.3)
IX-X cranial nerves only	4/24 (16.7)
VII-IX-X cranial nerves	12/24 (50)
Dysautonomia	22 (75.9)
Arrhythmia	9 (31)
Labile blood pressure	19 (65.5)
Diaphoresis	4 (14)
Urinary retention	10 (34)
Ileus	9 (31)
Intensive care unit admission	20 (69)
Respiratory failure	14/20 (70)
Invasive mechanical ventilation	9/14 (64.3)
Noninvasive mechanical ventilation	4/14 (28.6)
Both	1/14 (7.1)
Lumbar puncture n = 11	13 (45)
Cerebrospinal fluid proteins raised	10/11 (91)
Albuminocytologic dissociation	10/11 (91)
Pleocytosis	0 (0)
Electrophysiological findings	
Acute inflammatory demyelinating polyneuropathy	16/27 (59.2)
Acute motor axonal neuropathy	7/27 (25.9)
Acute motor sensory axonal neuropathy	4/27 (14.8)
Treatment after symptoms	
Less than 7 days	16 (55.1)
More than 7 days	7 (24.1)
None	6 (20.7)
Disability (by Hughes' functional scale)	
1	4/27 (14.8)
2	5/27 (18.5)
3	4/27 (14.8)
4	14/27 (51.8)
5	0 (0)
6	0 (0)

The patient with myeloradiculopathy was a 25-year-old woman with complaints of inability to walk associated with urinary retention, who 2 days before had fever, rash, arthralgia, conjunctivitis and diarrhea. Central nervous system examination revealed decreased reflexes in upper and lower limbs, decreased temperature sensations in neck, and abdominal zone with generalized weakness.

4. Discussion

Cúcuta belongs to one of the most endemic regions within Colombia for the transmission of ZIKV associated with the spread of *Aedes aegypti*, and provides a unique eco-epidemiological condition that helps to promote arbovirus transmitted diseases. Given the potential for ZIKV to spread globally, it is critical to characterize the transmission dynamics of the infection. The R_0 in Cúcuta ranged between 2.68 and 4.57. Our sensibility analysis disclosed a narrow range of R_0 estimates as compared with previous reports in different regions of the world (Fig. 7 and Table 7) [30–36]. R_0 indicates how many individuals (including both symptomatic and asymptomatic) are typically infected during an outbreak and is a function of both, disease and geographical setting and will thus vary among local environment, human behavior, vector abundance, and, potentially, interactions with other viruses [37]. Thus, these results should be interpreted with caution because R_0 is very sensible to generation time distribution assumption, as noted by Chowell et al. [24]. Moreover, there is considerable uncertainty about the distribution for ZIKV infection [32].

The AR corresponds to the fraction of the population that becomes infected, which is also an important concept in measuring the transmission of infectious diseases. In Cúcuta, we observed a complex interaction between age and gender on the AR by using a GAM modelling approach. Although marginal differences in the AR by gender have been reported [36] no consideration of the interaction with age has been analyzed. Young children (newborn) and women between 15 and 39 years old are at the highest risk of being infected (Fig. 3). It should be taken into account that the INS considered newborn and pregnant women as a population at risk; therefore, the AR in these groups could be overestimated by inclusion bias. Both R_0 and ARs could help assist with outbreak planning, assessment of potential countermeasures, and the design of studies to investigate putative associations between ZIKV disease and other conditions.

The maps provide relevant information about the critical sites for potential contamination and the relationship between the transmission of the ZIKV and the breeding sites of the mosquito [38] along with the shared incidence with cases of ZIKV disease and neurological outcomes such as GBS and other neurological syndromes. The interaction of the local ecology, including the vector, the natural history of ZIKV, and the population's susceptibility to infection are reflected in Fig. 8 [33,38–48].

The mounting evidence of neuro-pathological involvement following ZIKV infection has been generated by a number of case-control studies in addition to a number of ecological and epidemiological studies with one of the focus on defining the relationships, if any, between ZIKV infection and GBS [49]. GBS in Cúcuta during the ZIKV outbreak was slightly more prevalent in men than in women, in agreement with other reports [23]. The median lag time between ZIKV infection and the onset of neurological symptoms was 7 days, similar to the lag time reported in the French Polynesia study [50] and in another studies from Colombia [51,52]. ZIKV infection seems to trigger a rapid onset of neurological symptoms, in contrast to other infections where the symptoms develop between a period of 2 and 4 weeks [53]. This finding suggest two mechanisms by which ZIKV may trigger GBS: neurotoxicity (in those with an early presentation) and autoimmune mediated.

Clinically, patients had a monophasic and progressive disease, as previously described [22,23]. Cranial nerve involvement was observed in a high percentage (82.7%) of patients, in contrast with other studies [12], and it was associated with an elevated degree of disability after hospital discharge. Dysautonomia was observed in a much higher percentage (75.9%) than in previous reports in which such a condition has been reported in a range between 22% and 47.1% [54,55]. The lability in blood pressure was the most frequent dysautonomia in our GBS patients (65.5%), which is in contrast with a previous study that reports a maximum percentage of 10%, but even in this study this was the predominant manifestation reported [56]. The findings from the present study also suggest that not only is the appearance of arrhythmia the most ominous dysautonomia that contributes to a poor prognosis [22], but also the concomitance of other additional risk factors such as respiratory failure.

The recovery prognosis of GBS patients is generally favorable, however mortality rates range from 2 to 15%, and 15–20% of patients have persistent disability after 6 months [56,57]. Although no deaths were reported in the present study, the typical complications from GBS such as dysautonomia, ICU admission and

Table 4
General characteristics of patients with GBS associated with ZIKV (cases) and patients with only ZIKV infection (controls).

Characteristic	Cases (N = 29) N (%)	Controls (N = 74) N (%)	OR (95% CI)
Age (IQR)	42 (34–49)	37.5 (27–51.7)	–
Male	15 (52)	26 (35)	0.51 (0.20–1.22)
Scholarship (years)			
0	2 (7)	2/73 (3)	1.0
1–9 years	11 (38)	24/73 (33)	1.33 (0.32–14.07)
>9 years	16 (55)	47/73 (64)	1.84 (0.45–18.13)
Socioeconomic status			
1	12/28 (43)	11/73 (15)	1.0
2	12/28 (43)	36/73 (49)	2.76 (1.13–8.86)[a]
3	2/28 (7)	13/73 (18)	4.33 (1.22–28.19)[a]
4	2/28 (7)	13/73 (18)	4.33 (1.22–28.19)[a]
Social insurance			
Individual account	16 (55.2)	39/71 (55)	1.0
Social assistant system	10 (34.5)	22/71 (31)	0.8 (0.35–2.27)
Others	3 (10,3)	10/71 (14)	–
Occupation			
Manual exclusive	15 (51.7)	15/72 (20.8)	1.0
Intellectual exclusive	3 (10.3)	11/72 (15.3)	2.57 (0.82–13.16)
Mixed	2 (6.9)	18/72 (25)	5.62 (1.65–33.05)[b]
Housewife	6 (20.7)	14/72 (19.4)	0.36 (0.09–3.01)
Unemployed	1 (3.4)	1/72 (1.4)	0.46 (0.09–10.69)
Retired	1 (3.4)	2/72 (2.8)	0.93 (0.19–14.21)
Student	1 (3.4)	11/72 (15.3)	5.15 (1.21–48.3)[c]
Toxics			
Organic solvents	4 (14)	11/73 (15)	0.79 (0.29–3.13)
Hair dye	9 (31)	31/73 (42)	0.61 (0.23–1.51)
Pesticides	2 (7)	6/73 (8)	0.68 (0.20–4.33)
Combustible	1 (3)	2/73 (3)	0.81 (0.19–11.9)
Previous infectious (perceived morbidity)			
Upper respiratory infection	2 (7)	8 (11)	0.52 (0.16–3.12)
Pneumonia	1 (3)	4 (5)	0.48 (0.12–5.05)
Hepatitis	3 (10)	5 (7)	1.27 (0.40–6.85)
Dengue virus	2 (7)	11 (15)	0.37 (0.11–2.11)
Chikungunya virus	19 (65.5)	42 (57)	1.43 (0.59–3.64)
Zika symptoms			
Fever	22 (76)	50/73 (68)	1.42 (0.54–4.09)
Rash	25 (86)	61/73 (83.5)	0.96 (0.35–3.71)
Arthralgia	23 (79)	56/73 (77)	1.14 (0.41–3.57)
Conjunctivitis	19 (65.5)	40/73 (55)	1.55 (0.64–3.94)
Diarrhea	13 (45)	26/73 (36)	1.46 (0.6–3.54)
Previous vaccination			
Yellow fever	11 (38)	16 (22)	2.19 (0.84–5.65)
Influenza	4 (14)	11 (15)	0.80 (0.29–3.18)
Others	3 (10)	16 (22)	0.43 (0.09–1.47)
Comorbidities			
Autoimmune disease	0 (0.0)	4/73 (5)	0 (0.01–5.01)
Cardiovascular disease	2 (7)	15/73 (20.5)	2.25 (0.08–1.40)
Metabolic-Renal disease	0 (0.0)	12/73 (16)	0 (0.005–1.45)
Pulmonary disease	0 (0.0)	4/73 (5)	0 (0.01–5.01)

[a] P for trend < 0.02.
[b] P for trend = 0.003.
[c] P for trend = 0.01.

respiratory support had a higher prevalence than in other studies [54–58], indicating that GBS associated with ZIKV has an aggressive clinical course.

Most of the patients presented the AIDP sub-phenotype which is in agreement with previous reports establishing the AIDP sub-phenotype as the most common one in Colombia and South America as well as in Europe and the United States [15,59,60]. In contrast, the acute motor axonal neuropathy sub-phenotype is predominant in Asia, and it has been highly associated with a previous infection with *C. jejuni* [23,61]. Noteworthy, AIDP was the GBS clinical sub-phenotype with better prognosis with an observed low rate of ICU admission. Our results indicate that the high load of infections, mainly *M. pneumoniae* exposure, is a risk factor to develop GBS after ZIKV infection. Although a relationship between *M. pneumoniae* infection and GBS has been previously reported [62], the role of previous infection with *M. pneumoniae* in the development of GBS associated with ZIKV deserves further investigation.

A number of other neurological syndromes different from GBS associated with ZIKV infection were observed, as have been reported [63–65]. In our cases, myelitis may have had a para-infectious etiology, indicating that the neurological injury may be associated with a direct microbial infection followed by an immune-mediated damage, or a remote infection followed by a systemic response that induces neural injury [66].

All patients with transverse myelitis had hyper-reflexia, indicating a central (white matter dysfunction) rather than a peripheral cause of muscle weakness [67,68]. The presence of a defined thoracic sensory level was observed in all the patients, as has been reported. Autonomic signs were observed, including arrhythmia

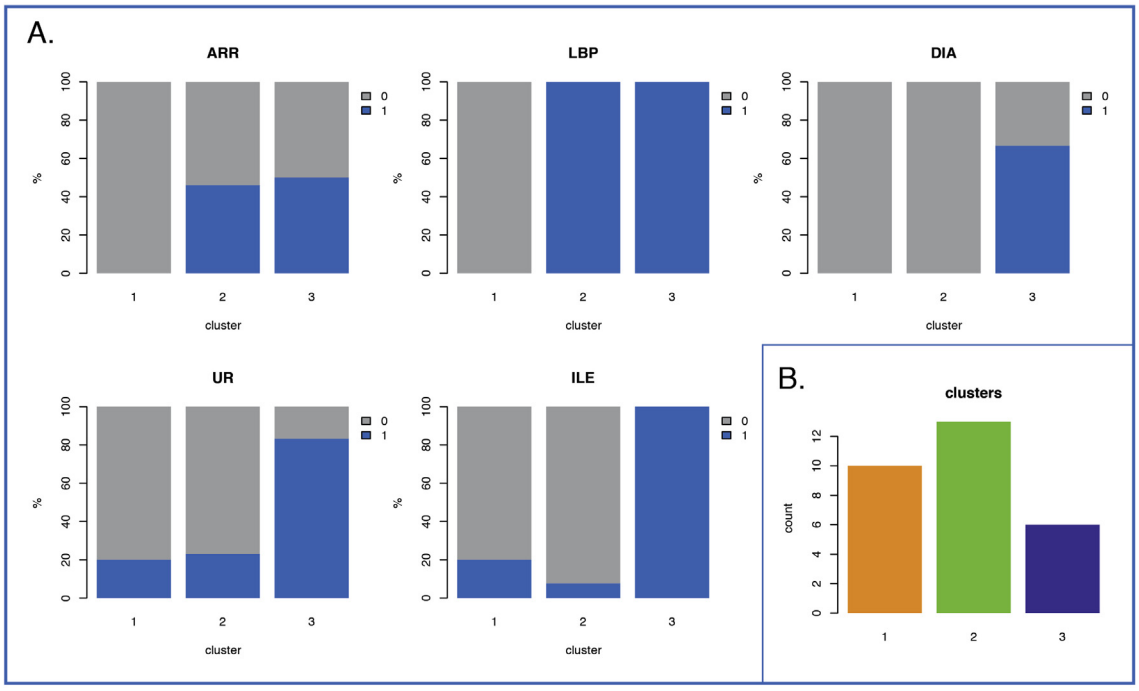


Fig. 6. Mixed cluster analysis of Guillain-Barré patients. Patients were grouped into three groups using mixed cluster analysis in order to evaluate the behavior of the 5 main dysautonomia studied. Panel A shows the relative frequencies of each dysautonomia for cluster 1 to 3. ARR (Arrhythmia), LBP (Labile blood pressure), DIA (Diaphoresis), UR (Urinary retention), ILE (Ileus). 0: No, 1: Yes. Panel B shows the absolute frequencies of each cluster.

Table 5
Infectious profile in patients with other neurological syndromes.

	ZIKV		DENV		CHIKV		*C. jejuni*	*M. pneumoniae*		CMV		EBV	
	IgM	IgG	IgM	IgG	IgM	IgG	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Transverse myelitis													
Patient 1	−	+	−	+	−	−	+	−	−	−	+	−	+
Patient 2	−	+	−	+	−	+	−	+	+	−	+	−	+
Patient 3	−	+	−	+	−	−	−	−	−	−	+	−	+
Patient 4	−	+	−	+	−	−	−	−	+	−	+	−	+
Patient 5	−	+	−	+	−	−	+	−	+	−	+	−	+
Patient 6	−	+	−	+	−	−	−	−	−	−	+	−	+
Encephalitis													
Patient 1	−	+	−	+	−	+	−	−	+	−	+	−	+
Patient 2	−	+	−	+	−	+	−	−	+	−	+	−	+
Patient 3	−	+	−	+	−	−	−	−	−	−	+	−	+
Peripheral facial palsy													
Patient 1	−	+	−	+	−	+	+	−	−	−	+	−	+
Patient 2	−	+	−	+	−	−	−	−	−	−	+	−	+
Patient 3	−	+	−	+	−	+	−	−	+	−	+	−	+
Myeloradiculopathy	−	+	−	+	−	+	−	−	−	−	+	−	+

and blood pressure lability [68], which are unusual autonomic signs in transverse myelitis [69,70]. So far our patients with transverse myelitis have presented with a monophasic disease, similar to what is described elsewhere [66]. In order to evaluate the likelihood of presenting a recurrence, the presence of auto-antibodies, including IgG anti-aquaporin 4 (AQP4) and anti-Ro antibodies, and evaluation of magnetic resonance imaging (MRI) was assessed [71]. Anti-Ro and AQP4 antibodies were not observed, but 3 of 4 patients in whom a MRI was performed showed evidence of 3 vertebral segment involvement (a complete or longitudinally extensive transverse myelitis). Thus, they may have a low risk of recurrences consistent with relapsing myelitis, neuromyelitis optica or developing multiple sclerosis [66,72,73].

Myelopathies can be subdivided into compressive and non-compressive causes. The non-compressive myelopathies can be classified as ischemic, para-neoplastic, infectious, or systemic autoimmune diseases [74]. Among the latter, transverse myelitis can be associated with systemic lupus erythematosus [75], Sjögren's syndrome [76] and the antiphospholipid syndrome (APS) [74]. In one of our patients, anti-phospholipid antibodies were present in two samples obtained 12 weeks apart (patient 2, data not shown), suggesting that she could develop APS in the future.

Even though the main neurological complications of ZIKV in adults involve the peripheral nervous system, 3 cases of central nervous system involvement (i.e., encephalitis) were observed, as has been previously reported [64,77,78]. These 3 patients had a history of other arbovirus infections such as DENV and CHIKV [14]. Of these 3 cases 2 were admitted to the ICU and required mechanical ventilation. The imaging findings were normal [78,79], in spite of an active inflammation characterized by pleocytosis and a

Table 6
Clinical features of patients with transverse myelitis.

Variable	N (%) or median (IQR)
Age (years)	22.5 (17.5–32)
Male	4 (66.7)
Lag between ZIKV and neurological disease (days)	32 (13–96)
Paresthesia	4 (66.7)
Symmetric weakness	4 (66.7)
Lower limb weakness	5 (83.3)
Trunk weakness	2 (33.3)
Hyper-reflexia	6 (100)
Defined sensory level	6 (100)
Autonomic signs	3 (50)
Urinary retention	3 (50)
Ileus	3 (50)
Arrhythmia	2 (33.3)
Blood pressure lability	1 (16.7)
Intensive care unit admission	2 (33.3)
Respiratory failure	0 (0)
Lumbar puncture	3 (50)
Pleocytosis	2/3 (66.7)
Magnetic resonance imaging	4 (66.7)
Gadolinium-enhancing cord lesion	4/4 (100)
Treatment after symptoms	
Less than 7 days	5 (83.3)
More than 7 days	0 (0)
No data	1 (16.7)
IgG anti-aquaporin 4 antibodies[a]	0 (0)
Treatment	
None	0 (0)
Corticosteroids	5 (83.3)
No data	1 (16.7)

[a] By ELISA (ElisaRSR™ AQP4 Ab, RSR Limited, Pentwyn, Cardiff, U.K.).

high protein concentration and normal glucose in the cerebrospinal fluid. These findings highlight the neurotropic properties of ZIKV [80,81], that has been implicated as the cause of direct damage to neuronal tissues which could lead to exposure of the immune system to neo-antigens and result in auto-immune mediated response [77].

Three cases of facial palsy were also observed, a rare complication of ZIKV infection. In fact, according to Polynesia Epibulletin, of the 70 cases that presented neurological complications, one case of facial palsy following ZIKV infection was reported [82]. Lastly, one of our patients presented with myelo-radiculopathy, which to the best of our knowledge is the first such case reported following ZIKV infection.

We acknowledge the study limitations, including the use of a surveillance system in which underreported cases are not identifiable. Also, difficulties to measure incidence and to accurately interpret associations, the time elapsed between the neurological symptoms and blood sampling as well as the bias due to low response, are all potential shortcomings. Nevertheless, efforts were made to include most of the patients with neurological syndromes registered to the SIVIGILA. Nonetheless there was a significant increase of severe GBS during the ZIKV outbreak in Cúcuta. Low socioeconomic status and a high load of previous infection (i.e. *M. pneumoniae*) were the main risk factors associated with GBS development. The mechanisms of interaction between infectious factors and host leading to autoimmune response in GBS are not completely known [23]. Therefore, in the cases of GBS it is important to distinguish between ZIKV neurotoxicity, known since the first description of the virus [83], and an autoimmune response, generated through molecular mimicry and other mechanisms [84], and favored by the presence of current or previous infections [85].

The hallmark of autoimmunity is the dysregulation of the immune system, especially T and B cells recognizing self-antigens as foreign. The ability of T cells to evade central and peripheral mechanisms of tolerance is evident by the large number of T cell mediated autoimmune diseases. There has been an enormous effort directed at trying to understand the mechanisms that lead to loss of tolerance, including genetic predisposition and environmental factors in virtually every autoimmune disease [86–89]. Activation of an autoimmune response could be modulated by viral infection through a variety of mechanisms such as molecular mimicry and cross-reactivity, cross presentation, epitope spreading, bystander involvement, heterologous viral memory and polyclonal stimulation [85]. Molecular mimicry has been attributed either to the similarity between pathogen-derived antigens and self-antigens, or a non-specific innate immune activation producing loss of tolerance and development of an immune response against auto-antigens. Clearly there are a variety of mechanisms

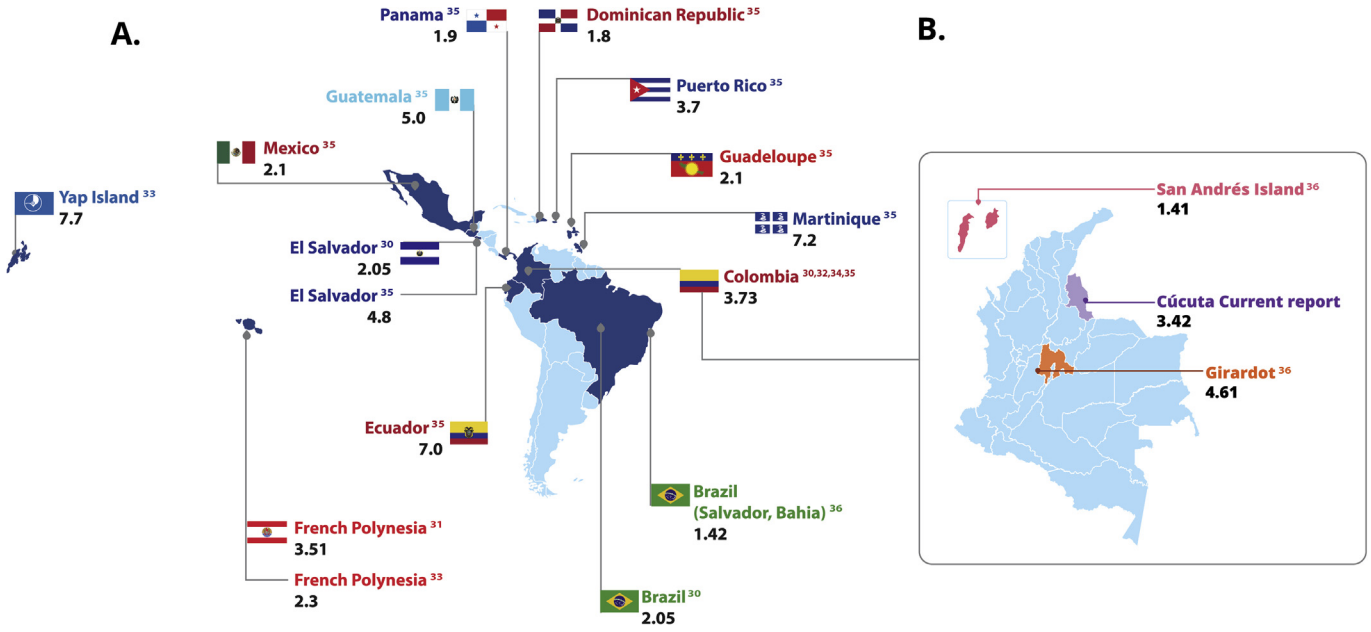


Fig. 7. Basic reproduction numbers. A. The Pacific and the Americas, B. Colombia.

Table 7
Basic reproduction number for Zika infection (Review of the literature).

Author, year	Place	R0 (95% CI)	Method	Comments
Gao et al., 2016 [30]	Brazil, Colombia, and El Salvador	2.05 (95% IC: 0.523–6.300)	Mathematical method based on Ross-Macdonald malaria model	Data up to February 27, 2016.
Kucharski et al., 2016 [31]	French Polynesia	Mean 3.51 (Range 2.6–4.8)	Compartmental mathematical model	GBS incidence 16.4 (95% CI: 11.5–21.4) per 100,000 cases.
Majumder et al., 2016 [32]	Colombia	Mean 3.26 (Range 1.91–5.05)[a] Mean 5.36 (Range 2.52–9.63)[b]	IDEA model	
Nishiura et al., 2016 [33]	Yap Island	Mean 7.7 (Range 2.8–12.5)	MLE and EEGR model	2007 data.
Nishiura et al., 2016 [33]	French Polynesia	Mean 2.3 (Range 1.5–3.1)	MLE and EEGR model	Data 2013–2014.
Nishiura et al., 2016 [34]	Colombia	Mean 4.8 (Range 3.0–6.6)	MLE and EEGR model	Until March 12, 2016.
Rocklöv et al., 2016 [35]	Guadeloupe	2.1	MLM	R_0 predictive model is proposed in Europe.
Rocklöv et al., 2016 [35]	Martinique	7.2	MLM	
Rocklöv et al., 2016 [35]	Dominican Republic	1.8	MLM	
Rocklöv et al., 2016 [35]	Mexico	2.1	MLM	
Rocklöv et al., 2016 [35]	Guatemala	5.0	MLM	
Rocklöv et al., 2016 [35]	Panama	1.9	MLM	
Rocklöv et al., 2016 [35]	Puerto Rico	3.7	MLM	
Rocklöv et al., 2016 [35]	El Salvador	4.8	MLM	
Rocklöv et al., 2016 [35]	Colombia	3.2	MLM	
Rocklöv et al., 2016 [35]	Ecuador	7.0	MLM	
Rojas et al., 2016 [36]	Girardot, Colombia	4.61 (95% IC 4.11–5.16)	MLM	R_0 female: 1.28 (1.17–1,40). R_0 0–19 year: 0.37 (0.33–0.42). R_0 > 50 years: 0.46 (0.41–0.52). Attack rate: 18.43 per 1.000 residents.
Rojas et al., 2016 [36]	San Andrés Island, Colombia	1.41 (95% IC: 1.15–1.74)	MLM	R_0 female: 1.71 (1.50–1.95). R_0 0–19 years: 0.86 (0.74–0.99). R_0 > 50 years: 0.74 (0.63–0.88). Attack rate: 12.13 per 1000 residents.
Rojas et al., 2016 [36]	Salvador, Brazil	1.42 (95% IC: 1.35–1.49)	MLM	Attack rate of 5.5 cases per 1000 Salvador residents.
Current report	Cúcuta, Colombia	Mean 2.034 (Range 1.54–2.52)	MLE and EEGR model	Sex-specific attack rates: 26.69 per 1000 females and 9.07 per 1000 males.

Abbreviations: IDEA: Incidence Decay and Exponential Adjustment; GBS: Guillain-Barré syndrome; MLE: maximum likelihood estimation; EEGR: Early exponential growth rate; MLM: Maximum likelihood methods.
[a] Using the digital (smoothed HealthMap) cumulative case counts.
[b] Using Colombian National Institute of Health data.

that involve not only T and B cell regulatory factors, but also components of innate immunity [90–92]. In GBS context, the primary target for immune-related injury is directed at Schwann-cell components or membranes on the nerve axon (the axolemma) [23]. Previous infectious agents have been described to trigger this neuropathology and suggest a causal relationship between these and the autoimmune response associated with GBS such as infections due to *C. jejuni*, *M. pneumoniae*, EBV and CMV. Molecular mimicry between antigens of ZIKV and host tissues are an attractive hypothetical mechanism for the triggering of neurologic disorders bolstered by a previous infection (i.e., *M. pneumoniae*).

Although the end of the global health emergency over the spread of the ZIKV was declared by the WHO on November 18, 2016, the global risk assessment has not changed [93]. ZIKV

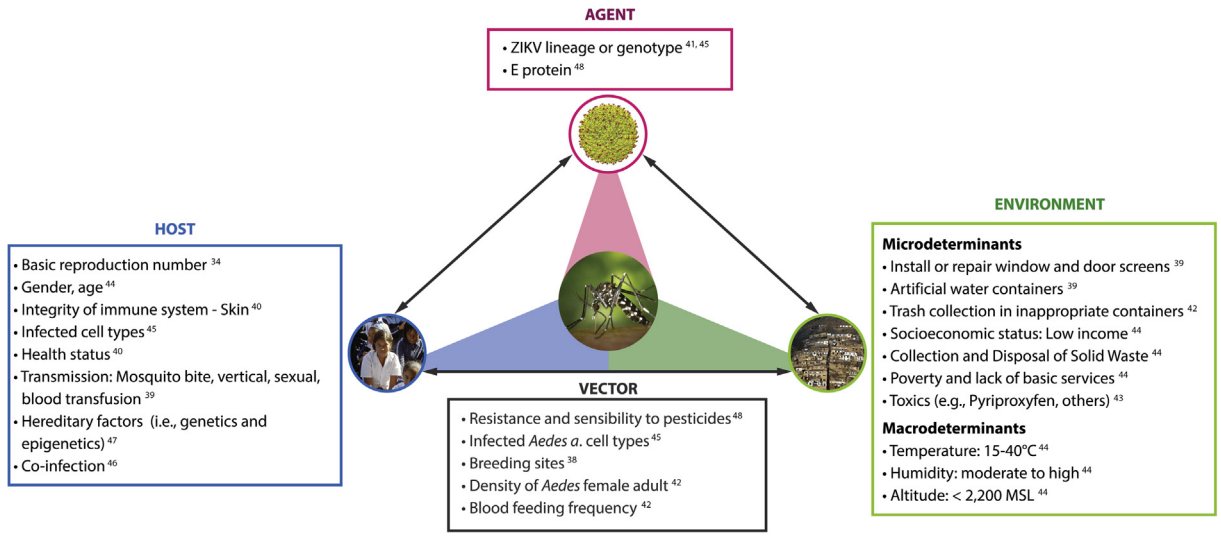


Fig. 8. The Zika virus epidemiologic triade. The most productive breeding places of *Aedes aegypti* in Cúcuta are unprotected water containers as those located in homes, such as water storage tanks used in homes during the dry and rainy seasons and public spaces that promote water stagnation during the rainy season.

continues to spread geographically to areas where competent vectors are present. Although a decline in cases of ZIKV infection has been reported in some countries, or in some parts of countries, vigilance needs to remain high. It is necessary to create more aggressive and efficient control systems for vector-borne viral diseases affecting humans. Primary health strategies should be enforced, beyond just educating people, and also provide essential health care, through means accessible to all individuals and families in the community. Further studies and continued efforts from the government aimed at eradicating the vector are warranted. We suggest that ZIKV infection is an ideal system in which a systems-biology type of analysis [94] would be appropriate to identify host factors, concurrent health conditions that influence susceptibility and outcome, and finally, of course, effector pathways.

Competing interest

None.

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