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Surveillance of microcephaly and selected brain anomalies in Argentina: Relationship with Zika virus and other congenital infections

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Background: Zika virus (ZIKV) vertical transmission may lead to microcephaly and other congenital anomalies. In March and April 2016, the first outbreak of ZIKV occurred in Argentina. The objective was to describe the surveillance of newborns with microcephaly and other selected brain anomalies in Argentina, and evaluation different etiologies.

Methods: Participants were enrolled between April 2016 and March 2017.

Cases: newborns from the National Network of Congenital Abnormalities of Argentina (RENAC) with head circumference lower than the 3rd percentile according to gestational age and sex, or selected brain anomalies. Blood and urine samples from cases and their mothers were tested for ZIKV by real-time polymerase chain reaction (RT-PCR), antigen-specific Immunoglobulin M (MAC-ELISA) and plaque-reduction neutralization test ($PRNT_{90}$). Toxoplasmosis, rubella, herpes simplex, syphilis, and cytomegalovirus (CMV) infection were also tested.

Results: A total of 104 cases were reported, with a prevalence of 6.9 per 10,000 [95% confidence interval (CI): 5.7–8.4], a significant increase when compared with the data prior to 2016, Prevalence Rate Ratio 1.7 (95% CI 1.2–2.3). In five cases positive serology for ZIKV (IgM and IgG by PRNT) was detected. The five cases presented microcephaly with craniofacial disproportion. We detected four cases of CMV infection, three cases of congenital toxoplasmosis, two cases of herpes simplex infection, and one case of congenital syphilis.

Conclusion: The prevalence of microcephaly was significantly higher when compared with the previous period. The system had the capacity to detect five cases with congenital ZIKV syndrome in a country with limited viral circulation.

KEYWORDS

Argentina, congenital anomalies, congenital infections, microcephaly, Zika virus

1 | INTRODUCTION

Zika virus (ZIKV) is a *Flavivirus* transmitted mainly by *Aedes* mosquito species (Haddow, Williams, Woodall, Simpson, & Goma, 1964). Its first isolation was in 1947 from a rhesus monkey in the Zika forest, Uganda (Dick, Kitchen, & Haddow, 1952). Subsequently, sporadic human infections were reported in Africa and Asia, and the first documented ZIKV outbreak occurred in Yap State, Federated States of Micronesia in 2007 (Duffy et al., 2009). In 2013, the infection spread to other regions of Oceania (Cao-Lormeau & Musso, 2014). In the Americas, the first confirmed case of ZIKV infection was reported in Brazil in May 2015 (Kindhauser, Allen, Frank, Santhana, & Dye, 2016). Six months later, a remarkable increase in the number of infants born with congenital microcephaly was observed in northeast Brazil (Recife, Pernambuco state) coinciding in time and space with the ZIKV outbreak (Kleber de Oliveira et al., 2016). This event led to the World Health Organization (WHO) declaring the Public Health Emergency of International Concern (PHEIC), and recommending intensifying surveillance and investigation activities on the unusual increase of cases of microcephaly and other neurological anomalies and their possible association with ZIKV (World Health Organization [WHO], 2016a,b). The ZIKV also quickly spread to other countries in South America, Central America, and the Caribbean. In Brazil there were more than 200,000 infected people and more than 2,000 confirmed congenital syndrome associate with ZIKV infection during 2016 (Pan American Health Organization [PAHO], 2016a,b).

ZIKV can be contracted mainly through the bite of an infected mosquito or through a sexual relationship (McCarthy, 2016; Musso et al., 2015). The virus can also be transmitted by prenatally passing from mother to fetus (Calvet et al., 2016). Prenatal ZIKV infection has been linked to adverse pregnancy and birth outcomes, most notably microcephaly and other major brain anomalies. Microcephaly has been the clinical sign initially detected in this embryopathy but the phenotype has now been extended to other anomalies (Rasmussen, Jamieson, Honein, & Petersen, 2016). However, microcephaly is a clinical sign that can be detected at birth and it could be used as a sentinel sign. At the same time the etiology of microcephaly is heterogeneous since there is a broad range of genetic and environmental factors involved.

In Argentina, a neighboring country of Brazil, there is a wide distribution of the vector *Aedes aegypi* and outbreaks of several flaviviruses have been detected: Dengue (DENV), St. Louis Encephalitis Virus (SLEV), West Nile Virus (WNV), and Selvatic Yellow Fever (YFV) viruses in different parts of the country (Fabbri & Morales, 2016; Ministerio de Salud de la Nación Argentina [MSNA], 2015). In March and April 2016, the first outbreak of ZIKV by vector transmission occurred in the northern province of Tucumán, with 25 confirmed cases. In the same year, imported cases were detected; however, there were no other autochthonous cases (MSNA, 2016a). In the year 2017, new outbreaks of the infection have been reported in three northern provinces of the country (Salta, Formosa, and Chaco), starting in February (MSNA, 2017a). ZIKV epidemics also affected bordering countries (Brazil, Bolivia, and Paraguay).

The objective of this study was to describe the surveillance of newborns with microcephaly and other selected brain anomalies in Argentina, and the evaluation of different etiologies.

2 | MATERIALS AND METHODS

This study was carried out integrating the actions of the National Network of Congenital Abnormalities Argentina (RENAC, from its Spanish initials) under the National Center of Medical Genetics (CENAGEM, from its Spanish initials), the National Institute of Human Viral Diseases, "Dr. Julio I. Maiztegui" (INEVH, from its Spanish initials) and the National Institute of Infectious Diseases "Dr. Carlos Malbrán" (INEI, from its Spanish initials), the three institutions belonging to the National Administration of Laboratories and Health Institutes (ANLIS, from its Spanish initials) under the National Health Ministry of Argentina.

RENAC was the primary source for clinical detection of cases. This is a hospital-based surveillance system for newborns with major morphological congenital anomalies (CAs) (Groisman, Bidondo, Barbero, & Liascovich, 2016; Groisman et al., 2013). RENAC includes the main maternity hospitals of the 24 provinces of the country, covering approximately 300,000 births per year, which represents 43% of births of Argentina. RENAC coverage is higher in the public subsector than in the private and social insurance

sectors. The case definition includes all live births and stillbirths weighting 500 g or more, with major morphological CAs, whether external or internal, identified from birth until hospital discharge, and detected by physical examination or complementary tests, surgical interventions or autopsy. Because in Argentina elective termination of pregnancy for fetal anomaly (ETOPFA) is illegal, the RENAC does not include it. Every month, the participating hospitals retrieve all cases affected with CAs and load the data into a digital file, including the total number of live births and stillbirths. This report contains a verbatim description of the CAs observed on the affected newborn, along with a core set of variables. The RENAC coordination reviews the quality of descriptions and compliance with the inclusion criteria, and it checks if the information provided is incomplete or not clear. CAs coding is performed by geneticists of the coordination using the International Classification of Diseases 10th Revision (ICD-10), adapted by the Royal College of Pediatrics and Child Health. Following the analysis, surveillance information is disseminated to participating hospitals, health authorities and published on-line.

For the present study, an enhanced surveillance of cases with microcephaly and brain anomalies was carried out from April 2016 to March 2017. Participating hospitals were required to report each case with microcephaly or selected brain anomalies (cerebral atrophy, cerebellar atrophy, cerebral calcifications and hydrocephalus ex-vacuo [damaged brain matter shrink sandis surrounded by fluid]) within the first 48 hours of life. Microcephaly was defined as the presence of a head circumference (HC) smaller than the 3rd percentile for gestational age and sex according to INTERGROWTH-21st Project charts (Villar et al., 2014). Besides the core set of variables routinely reported for each case, an extended group of variables were included for cases with microcephaly and brain anomalies, and peripheral blood and urine samples from the newborns and their mothers were obtained to determine ZIKV and other congenital infections.

The INEVH coordinates the National Network of Laboratories for the diagnosis of Dengue and other Arboviruses. Since 2015 they have initiated laboratory surveillance for ZIKV in the country. For this study, the INEVH, carried out different methodologies for detection of ZIKV infection, like following the recommendation of the National Health Ministry of Argentina and international organizations (MSNA, 2016b,c; PAHO, 2106c; WHO, 2016c). Viral genome detection by real-time reverse transcription-polymerase chain reaction (rRT- PCR) specific for ZIKV was performed in the urine samples of the newborns and their mothers (Lanciotti et al., 2008). Serum samples from mothers and newborns were processed by serological techniques: in house antigen-specific Immunoglobulin M (IgM) enzyme-linked immunosorbent assay (MAC-ELISA) for ZIKV (Martin et al., 2000) and 90% plaque reduction neutralization test ($PRNT_{90}$) with a panel of flaviviruses composed of the four DENV serotypes (1, 2, 3, 4), ZIKV, SLEV, WNV, and YFV, performed on VERO C76 cell line. The $PRNT_{90}$ study was performed with the objective of detecting neutralizing Immunoglobulin G (IgG) antibodies and evaluating serological crosses within the *Flaviviridae* family (Russell, Nisalak, Sukhavachana, & Vivona, 1967).

In addition, tests were conducted to detect other congenital infections, including Toxoplasmosis, Rubella, Herpes simplex, shyphilis, and Cytomegalovirus (CMV). Enzyme immunoassay was performed for the detection of specific antibodies. Detection of the specific genome for each pathogen was performed by molecular biology techniques such as nested-PCR. These tests were performed by the INEI group.

The prevalence of cases was calculated as the quotient between the number of newborns and stillborns with microcephaly and other selected brain anomalies, and the total number of newborns and stillborns. The 95% confidence interval (CI) of prevalence was calculated according to the Poisson distribution, using the exact method. The prevalence observed from April 2016 to March 2017 was compared with the previously reported in the period 2009–2015 through the computation of the prevalence rate ratio and its 95% CI. The same analysis was performed for each clinical presentation of microcephaly. To assess trends during the enhanced surveillance period, we used the method of Cumulative Sums (CUSUM) to detect monthly increases in frequency or epidemic peaks, regardless of sample size. This method is a way to determine how the observed number of cases value deviates from an expected value using an expected threshold, determining the acceptable and unacceptable value of this deviation and visualizing the results in a graph (Chen, 1987). The expected threshold value (H) is calculated as the number of cases in a time period, plus the number of cases in the previous period, less a constant (K). This computation requires the establishment of certain a priori parameters: the baseline rate, the excess of cases that must occur to cross the threshold, the alpha error (number of periods that must elapse before giving a false alarm) and the beta error (number of periods it takes to detect a true alarm). Surpassing the threshold value raises an alarm of excess of cases. The following parameters were considered for the analysis: baseline prevalence 4 per 10,000 (according to 2009–2015 period); a prevalence greater than 6 per 10,000 as excess; $K = 5.571$; threshold value $H = 10.4$.

In order to assess the heterogeneity among the 24 provinces during the study period, a random effects meta-analysis of prevalence was performed, considering each province as a different study. A comparison between provinces with sustained transmission of dengue in 2016 (Buenos Aires, Ciudad de Buenos Aires, Córdoba, Entre Ríos, Santa Fe, Chaco, Corrientes, Formosa, Misiones, Jujuy, Salta, Santiago del

TABLE 1 Clinical characteristics of newborns with microcephaly and other selected brain anomalies, according different periods

Period	Outcome of pregnancy		Sex ratio (M:F)	Clinical presentation		
	Newborn *N* (%)	Stillbirth *N* (%)		Isolated *N* (%)	Multiple *N* (%)	Syndrome *N* (%)
2009–2015	289 (95.4%)	14 (4.6%)	0.7	135 (44.6)	140 (46.2)	28 (9.2)
2016–2017	104 (100)	0	1	57 (54.8%)	21 (20.2%)	26 (25.0%)

Estero, and Tucumán) and the provinces without sustained transmission (rest of the jurisdictions) was also carried out.

Cases were classified into three groups according to their clinical presentation: isolated cases (microcephaly and related cerebral abnormalities without other congenital anomaly), multiple congenital anomalies (MCA) (cases with other non-related congenital anomalies with an undefined etiology), and cases with microcephaly as part of syndromes (cases with other congenital anomalies with a defined etiology). Finally, we describe the clinical features and laboratory results for ZIKV positive cases.

3 | RESULTS

From April 2016 to March 2017, a total of 104 newborns with microcephaly or selected brain abnormalities (cerebral atrophy, cerebellar atrophy, cerebral calcifications, and hydrocephalus ex-vacuo) were detected in 150,057 births in the maternity hospitals of the RENAC. The total prevalence was 6.9 cases per 10,000 births (95% CI: 5.7–8.4). Of the 104 livebirths reported cases, 57 (54.8%) were isolated, 26 (25.0%) were syndromes, and 21 (20.2%) were cases with MCA (Table 1). Comparing this prevalence with the previous period 2009–2015 (4.1 per 10,000—CI%: 3.1–5.4), a statistically significant increase was detected with a Prevalence Rate Ratio (PRR) of 1.7 (95% CI: 1.2–2.3) (Figure 1). This increase was at the expense of isolated cases (PRR: 4.13 [CI 95%: 2.64–6.44]) and syndromic cases (PRR: 4.79 [CI 95%: 1.8–12.79]), while the MCA cases did not have a significant increase (PRR: 0.68 [CI 95%: 0.38–1.23]).

Variation in prevalence over the period of the study analyzed with the CUSUM method showed that the H-index exceeded the upper limit by August 2016 (Figure 2). The meta-analysis of random effects of prevalence showed a high heterogeneity between provinces, with a Cochrane Q value: 148.34, 15 degrees of freedom ($p < .05$). I^2 (%): 92.25 (Figure 3). In the jurisdictions with sustained transmission of dengue fever the prevalence was 6.9 per 10,000 (95% CI: 5.6–8.5); in those without sustained transmission, prevalence was 6.5 per 10,000 (CI: 95% 3.6–10.7). The PRR was not statistically significant (1.06; 95% CI 0.6–2.0).

Seventy-three (70.2%) of the 104 cases were studied for ZIKV. Of the remaining 31 cases, the cause of the microcephaly was attributed to a different specific diagnosis in 13 patients (monogenic: four cases—septooptic dysplasia, craniofrontonasal syndrome, microcephaly autosomal dominant, microcephaly autosomal recessive-; chromosomal: six (structural anomalies) cases and other noninfections teratogenic agents: three cases—alcohol, chemotherapy agent, and valproic cases-) and therefore they were not evaluated for ZIKV. Eighteen infant samples were not available for the study (predominantly patients who died soon after birth) (Figure 4).

ZIKV specific IgM was detected in 6.8% (5/73) sera samples from newborns. In a case (case #4), IgM was also detected in cerebrospinal fluid (CSF) and negative for DENV. Maternal serums were IgM positive at the time of delivery for the five cases. This group of infants and mothers

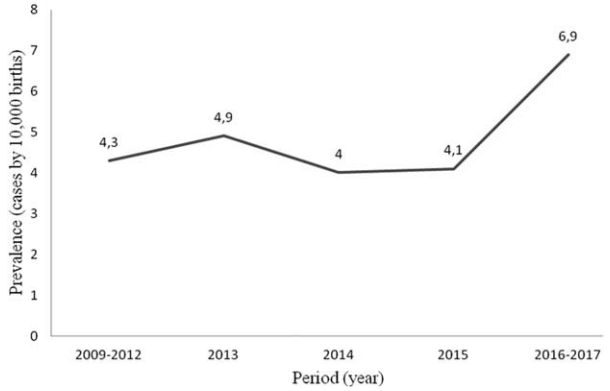

FIGURE 1 Annual prevalence of microcephaly and selected brain anomalies notified to National Network of Congenital Anomalies (RENAC) from November 2009 to March 2017

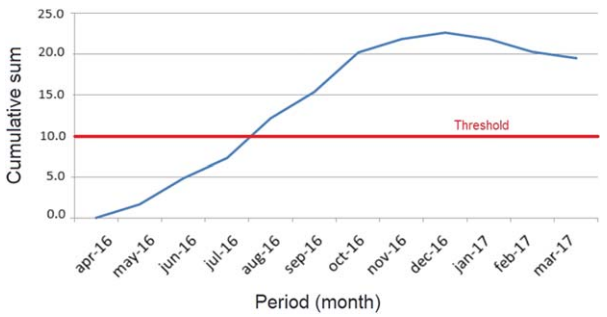

FIGURE 2 Cumulative sum from the newborns notified to National Network of Congenital Anomalies (RENAC) with microcephaly and/ or selected brain abnormalities from April 2016 to March 2017, according to month of birth

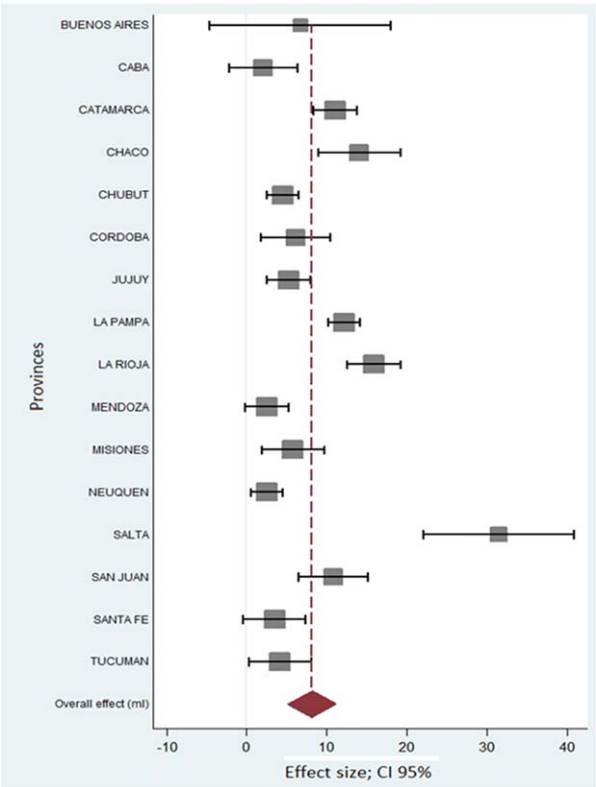

FIGURE 3 Prevalence of microcephaly and/ or selected brain anomalies reported to the National Network of Congenital Anomalies (RENAC) by province from April 2016 to March 2017

IgM positive, were also positive by PRNT90 for ZIKV (Titers ranging from 80 to > 1280). In 3/5 positive newborns, ZIKV titers were two dilutions higher than the other flaviviruses and allowed the viral identification. The two other infants, who had an epidemiological link with Bolivia, presented cross reactivity in $PRNT_{90}$ consistent with a secondary pattern immune response.

ZIKV IgM and ZIKV $PRNT_{90}$ were negative in 68 (93.2%, 68/73) cases and their mothers. Urine samples were negative for ZIKV genome in all of the cases included in this study (Tables 2 and 3).

Three cases were interpreted as imported since their mothers live in Argentina in regions without ZIKV vector transmission, but stayed in Santa Cruz de la Sierra, Bolivia —area with evidence of virus circulation—during the first trimester of pregnancy. The five cases were prenatally detected by ultrasonography during the second and third trimesters. This made possible to study mothers during gestation; in case #4, a real-time ZIKV genome was detected in a sample of amniotic fluid; however, none of the pregnant women had a positive RT-PCR neither in serum nor in urine (Table 2 and 3).

All cases presented a marked craniofacial disproportion, and neuroimaging compatible with ZIKV lesions; case #1 also presented distal arthrogryposis affecting the four limbs and a ring of constriction in the left leg (Table 3). In four of the five cases a karyotype was performed and the results were normal.

Regarding other congenital infections, four cases of CMV infection, three cases of toxoplasmosis, two cases of congenital Herpes simplex, and one case of congenital neurosyphilis were detected. No cases of rubella were detected.

4 | DISCUSSION

ZIKV congenital embryopathy is a new teratogenic disease. In February 2016, the WHO announced the PHEIC, based on the clusters of cases of newborns with microcephaly and others neurologic disorders from Brazil and Polynesia. Until 21 December 2017, 48 countries and territories in the

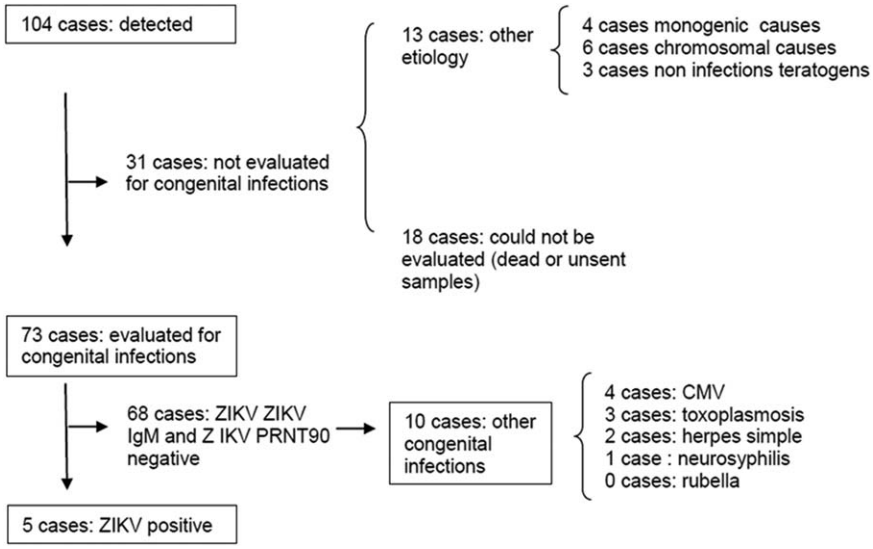

FIGURE 4 Cases detected with microcephaly and other selected brain anomalies reported to the National Network of Congenital Anomalies (RENAC), from April 2016 to March 2017

TABLE 2 Prenatal clinical characteristics and laboratory results of cases with ZIKV congenital syndrome reported to the National Network of Congenital Anomalies (RENAC) from April 2016 to March 2017

#Case	Jurisdictions, Birth month	Clinical signs in the pregnant woman	Travel outside the jurisdictions	Prenatal diagnosis										
				Fetal anomalies detected	ZIKV laboratory tests									
					qRT-PCR	MAC ELISA		PRNT90						
						DENV	ZIKV	DENV 1	DENV 2	DENV 3	DENV 4	YFV	SLEV	WNV	ZIKV
1	Tucumán, October 2016	no	no	Microcephaly, ventriculomegaly (ultrasonography 27 w.)	Negative urine	ND	Positive serum	10	<10	<10	<10	<10	<10	<10	≥**320**
2	Santa Fé, September 2016	maculopapular exanthema (13 w.)	no	Microcepahly (ultrasonography 34 w.)	Negative urine	ND	Border-line serum	10	20	10	<20	ND	ND	ND	≥ **40**
3	CABA, November 2016	Fever, maculopapular exanthema, articular oedema (8 w.)	Yes (Santa Cruz de la Sierra, Bolivia 1st trimester)	Microcephaly, ventriculomegaly periventricular calcifications. (ultrasonography 19 w.)	Negative urine and amniotic fluid	Negative serum	Positive serum	≥ 320	80	160	20	≥ 80	40	≥ 80	≥**320**
4	Córdoba, November 2016	Fever, maculopapular exanthema y conjunctivitis (9 w.)	yes (Santa Cruz de la Sierra, Bolivia 1st trimester)	Microlissencephaly, corpus callosum hypoplasia (MRI 3rd trimester)	Positive in amniotic fluid	Positive serum	Positive serum	≥ 160	≥ 160	≥ 160	80	≥ 80	20	20	≥ **160**
5	CABA, November 2016	Fever, articular oedema (12 w.)	yes (Santa Cruz de la Sierra, Bolivia 1st trimester)	Microcephaly (24 w)	NOT DONE										

ZIKV = Zika virus; CABA = Buenos Aires City; MRI = **magnetic resonance imaging; PCR =; ELISA IgM = antigen-specific Immunoglobulin M enzyme-linked immunosorbent assay; PRNT90 = plaque reduction neutralization test by 90% reduction in number of plaques; w = weeks; DENV = dengue virus; FAV = selvatic yellow fever viruses; SLEV = Saint Louis encephalitis virus; WNV = West Nile virus; ND = not determined.

TABLE 3 Clinical characteristics and laboratory results in newborns with ZIKV embryopathy reported to the National Network of Congenital Anomalies (RENAC) from April 2016 to March 2017

	Newborn phenotype						ZIKV laboratory tests											
							qRT-PCR	MAC ELISA		$PRNT_{90}$								
#Case	GE (w)	Weight (g)	HC	Medical Signs	Neuroimaging Finding	Autochthonous/ Imported		DENV	ZIKV	DENV 1	DENV 2	DENV 3	DENV 4	YFV	SLEV	WNV	ZIKV	Laboratory clasification
1	34	1940	31	microcephaly, arthrogryposis, constriction ring	ventriculomegaly	autochthonous	Negative urine	ND	Positive serum	<10	10	<10	<10	ND	ND	ND	**≥320**	ZIKV confirmed
2	39	3400	30	microcephaly	ventriculomegaly, cerebral atrophy, hypoplasia of the corpus callosum	autochthonous	Negative urine	Positive serum	Positive serum	<10	10	10	<10	<10	20	10	**≥320**	ZIKV confirmed
3	38	2800	29	microcephaly	ventriculomegaly, cerebral calcifications	Imported	Negative urine	Negative serum	Positive serum	≥80	≥80	≥80	20	ND	ND	ND	**≥80**	ZIKV Probable, FLAVIVIRUS CONFIRMED
4	41	3390	31	Microcephaly, opisthotonus, seizures	ventriculomegaly, cortical atrophy, lissencephaly, cortical-subcorticales calcifications	Imported	Negative ovulare membrane, umbilical cord and placenta	Negative serum and CSF	Positive serum and CSF	640	80	80	40	40	<40	<40	**≥1280**	ZIKV Probable, FLAVIVIRUS CONFIRMED
5	40	2350	27,5	microcephaly	ventriculomegaly, cerebral calcifications	Imported	The orine has not arrived	ND	Positive serum	<20	<20	<20	<20	<20	<20	<20	**≥640**	ZIKV confirmed

ZIKV = Zika virus; GE = gestational age; g = grams; HC = head circumference; PCR =; ELISA IgM = antigen-specific Immunoglobulin M enzyme-linked immunosorbent assay; $PRNT_{90}$ = plaque reduction neutralization test by 90% reduction in number of plaques; w = weeks; DENV = dengue virus; YFV = yellow fever viruses; SLEV = Saint Louis encephalitis virus; WNV = West Nile virus.

Americas have confirmed autochthonous, vector-borne transmission of ZIKV disease, while five countries have reported sexually transmitted ZIKV cases and 27 countries and territories in the Americas have reported confirmed cases of congenital embryopathy associated with ZIKV infection. Of the five countries bordering Argentina, three (Brazil, Bolivia, and Paraguay) have reported ZIKV transmission (PAHO, 2017).

In February 2016, Argentina reported the first autochthonous case of ZIKV infection, which was sexually transmitted (Central region, Córdoba province). In March and April 2016, the first argentine outbreak occurred (Northwest region, Tucumán province), with 33 cases with positive results for ZIKV (MNSA, 2016c). In 2017, new outbreaks of the infection started in other areas of the country. From January to August 2017, there were 250 confirmed autochthonous cases in the Northern provinces of Formosa, Salta and Chaco (MNSA, 2017b). Considering the study period, we did not expect to observe microcephaly or other brain anomalies caused by this recent outbreak.

All newborns with ZIKV laboratory positive results had a phenotype compatible with the congenital ZIKV embryopathy described on the published scientific literature (Moore et al., 2017). The RENAC prevalence of microcephaly showed a statistically significant increase between the previous period and the period of this study. Comparing both periods there was an increase of microcephaly with a specific known cause. In the second period there was a decrease in reported cases with multiple congenital anomalies and the cases with syndromes increased. The reason might be the improvement in the procedures for etiologic diagnosis (whether infectious, teratogenic, or genetic causes). The increase in the proportion of isolated affected cases might be probably due to an under-ascertainment in the previous period. Regarding the temporal time-trends during the period April 2016–March 2017, the CUSUM test showed an increase of prevalence since August 2016. This may be a real increase in the number of newborns affected. However, only five cases were confirmed with congenital ZIKV. Also, this increase might be attributable to the increased search for cases of microcephaly, arising from the alarm in the wake of the possible association with ZIKV (awareness effect). Significant heterogeneity was observed between provinces. These differences were possibly due to methodological factors and not to real differences in the geographical prevalence of microcephaly.

During 2016, Argentina had the greatest DENV outbreak in magnitude and geographical dissemination to date. The infection spread through 15 provinces of the center and north of the country, with 41,207 confirmed cases. This situation could have affected the detection of ZIKV infection both from the clinical point of view and from the serological studies (IgM cross reactivity) (MSNA, 2017b).

Laboratory testing for ZIKV has several limitations. It is an RNA virus only transiently present in body fluids; therefore, a negative PCR test result does not rule out infection. However, in some cases, prolonged detection of ZIKV RNA in serum obtained from pregnant women was also reported (until 80 days after symptom onset) (Meaney-Delman et al., 2016).

Serologic testing is affected by the timing of sample collection. ZIKV IgM antibodies typically become detectable within the first week after symptom onset and it is persistence time is not well known. These antibodies be difficult to interpret because of cross reactivity with other flaviviruses, especially in persons who were previously infected with or vaccinated against a related *Flavivirus*. The $PRNT_{90}$ is one of the most specific test available often used to define several serocomplexes of more closely related flaviviruses. To increase specificity, we selected a conservative threshold of 90% for PRNT. When individuals with no previous exposure to a flavivirus are infected with one of them, the etiologic agent can be accurately identified. Interpretation of heterotypic patterns is complex and fourfold difference in titers could be a limited criterion to provide distinction of the most recent infection (secondary pattern).

Recommended laboratory testing for congenital ZIKV infection include evaluation for ZIKV RNA in infant serum and urine, ZIKV IgM/IgG antibodies in serum, and the same studies in the mother. ZIKV RNA detection confirms diagnosis. If ZIKV IgM antibodies are detected in the infant with a negative PCR, the infant is considered to have probable congenital Zika virus infection. If neither ZIKV RNA nor ZIKV IgM antibodies are detected on samples obtained within the first few days after birth, congenital ZIKV infection is unlikely. However, samples are not always obtained so shortly after birth. Detection of IgG may reflect the passage of maternal antibodies and requires evaluation of the patient newborn at 18 months (Adebanjo et al., 2017).

In three cases of our study (cases #1, #2, and #5) serological tests confirmed the congenital infection by ZIKV. In cases #3 and #4, $PRNT_{90}$ did not differentiate a cross-reaction with other flaviviruses. However, these results should be analyzed by integrating clinical and epidemiological data. The presence of microcephaly or other brain abnormalities has not been associated with the other arboviruses. Additionally, mothers of case #3 and #4 had completed the first trimester of pregnancy in an area during a ZIKV outbreak.

The findings of this study are subject to some limitations. RENAC is hospital-based surveillance program, covering 43% of births of Argentina. Ninety-nine percent of births in Argentina occur in hospitals, and the inclusion of hospitals in the RENAC program is a gradual process that started in 2009. Thus, there may be a number of cases not detected and affected with congenital ZIKV. In addition, in our study

there were 18 newborns not available for studies but with a suspected phenotype, although none of them was born in a ZIKV outbreak area. The efforts should be intensified to increase the detection of cases, as well as the strategy to integrate the work of different programs and institutions that exist in the country.

For these reasons, there is probably an underascertainment of cases of congenital infection. In addition, although the case definition included newborns with microcephaly or specific brain anomalies (such as cerebral atrophy, cerebellar atrophy, cerebral calcifications, and hydrocephalus ex-vacuo), neuroimaging studies are not available in all hospitals. This could lead to under ascertainment of cases without microcephaly but with isolated brain anomalies. Despite these limitations, strengths of this study are the existence of a surveillance system of congenital anomalies with high coverage of births, previous the ZIKV emergency, and a laboratories network working coordinately with a large number of institutions in all the country's jurisdictions. Finally, no cases of rubella were detected, which is expected since this disease was eliminated from the Americas in 2009 (Andrus, Quadros, Solórzano, Periago, & Henderson, 2011). However, in other areas of the world rubella has not yet been eradicated, for example in sub-Saharan Africa and south Asia. Despite widespread vaccination programmes, an estimated 94% of pregnant women remain seronegative worldwide and therefore susceptible to infection (Pandolfi et al., 2017). This situation implies that even in areas where rubella has been eradicated, imported cases could be presented. While this situation persists we consider adequate to continue the epidemiological surveillance of suspected cases.

5 | CONCLUSION

Our study is the first investigation conducted in Argentina aimed to generate knowledge about the national situation on microcephaly and brain anomalies associated with ZIKV and other congenital infections. The joint work of RENAC, INEVH, and INEI, strengthened the surveillance system for the infectious etiology of microcephaly. Five cases with congenital ZIKV embryopathy were detected in Argentina, a country that had few isolated outbreaks of this disease. Cases with congenital ZIKV embryopathy detected had the phenotype described in the literature.

In our experience, the laboratory studies that mainly contributed to the diagnosis of ZIKV infection in newborns were MAC-ELISA and $PRNT_{90}$ since in the vast majority of cases there weren't viral detection.

Most birth defects surveillance systems started after the thalidomide embriopathy epidemic. These systems have enabled not only to establish epidemiological surveillance of congenital anomalies but also to contribute to the investigation of its causes. Since the ZIKV epidemic, several countries in the Americas started to develop birth defects surveillance programs. In Argentina, the RENAC has expanded its initial epidemiological objective to improve health care, health promotion, training, and evaluation of interventions. The detection of newborns with microcephaly and other brain abnormalities requires the multidisciplinary care of these patients in order to improve quality of life.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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