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8. Annexes

Annex 1

Table 1. Clinical data (mother and infant) of 13 children with presumed Zika virus congenital infection seen at General Regional Hospital Pascacio Gamboa, Tuxtla Gutierrez, 2016-2019

Patient No	Sex	DOB/End of Pregnancy	US-1	US-2	Mothers Age (years)	Trimester of pregnancy with rash		Phase of microcephaly diagnosis	Head circumference at birth (cm)	Z-score circumference at birth	Status/age at follow-up visit	Zika virus in CSF	Findings on US/TFUS/MRI/CT	Other findings (Histopathology, Labs) and follow-up
1	Girl	1/2018	29	-	19	Unknown	37	Pregnancy	28.5	-2.9	Died 59 days.	NA	Microcephaly (-4 SD), herniation of the orbital fat to the skull by US, giant occipital-parietal encephalocele, hypoplastic corpus callousum and absence of ventricles by MRI	Myelomeningocele, brainstem dysplasia, microcephaly
4	Girl	7/2018	12	13	32	Unknown	36	Birth	29.5	-3.2	Died 44 day.	NA	Microcephaly, hypoplastic left ventricle by US; hypoplastic corpus callosum by TFUS (Supp. Fig. 1 a-b)	Cleft palate, heart defects (hypoplastic right heart, tricuspid atresia and ventricular septum defect) by echocardiography (Supp. Fig. 1 c)
6	Girl	8/2016	21	27	41	First	33	Pregnancy	27	-3.6	Alive- 2 years	NA	Microcephaly detected by US at 28 weeks of gestation (Suppl. Fig. 2 a), fetal growth restriction and oligohydramnios by US; cysts germinal matrix, mild hydrocephalus, cystic encephalomalacia and cortical thinning by TFUS	At 32 months of age was able to stand but could not walk. Could babble but not speak more than 2 words.
7	Boy	8/2016	23	26	38	Unknown	39	Pregnancy	29	-3.6	Alive – 2years	NA	Hydrocephalus and microcephaly, right predominant ventriculomegaly by US (Suppl. Fig 3a); hydrocephalus (Evans 0.53, Suppl. Fig. 3 b); cysts germinal matrix and parenchymal cysts by TFUS (Suppl. Fig 3 c-d); basal ganglia calcifications, cortical thinning of both gyri and sulci by CT	Unable to walk, inconsolable crying, clubfoot, seizures, VIH (-), VDRL (-), CMV IgM (-), CMV IgG (+), Rubella IgG (+), Rubella IgM (-), Toxoplasma (-)

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8	Girl	10/2016	30	32	17	First	40	At birth	29	-3.8	Died at 1 year.	NA	Fetal growth retardation and severe oligohydramnios by US; microcephaly with absence of the septum pellucidum; intraventricular hemorrhage at the foramina of Monro by TFUS; hypoplasia of corpus callosum, brachycephaly, Dandy-Walker malformation, (Suppl. Fig. 4 a), thinning of sulci and gyri (Suppl. Fig. 4 b), in CT	Bronchopneumonia and disseminated intravascular coagulation on death certificate. Was able to say mom and dad at 8 months of age.
9	Girl	10/2016	5	11	21	Unknown	35	At birth	27	-3.3	Alive -2 years	NA	Severe oligohydramnios and enlargement of lateral ventricles by US (Suppl. Fig. 5); hypoplastic corpus callosum, lissencephaly and thin cortex by TFUS	Unable to walk, or seat, inconsolable crying, hip dysplasia, strabismus. VIH (-) VDRL (-)
11	Boy	2/2017	30	31	22	Unknown	40	Pregnancy	27	-4.6	Alive -2 years	NA	Fetal growth retardation and absence of midline structures and hypogenesis of frontal bone and nasal septum by US; hypogenesis of corpus callosum and basal ganglia calcifications by TFUS	Unable to walk, blind, deaf, able to babble at 4 months of age.
12	Boy	7/2017	23	30	30	Unknown	41	Pregnancy	28	-4.8	Alive	NA	Microcephaly (Suppl. Figure 6) and oligohydramnios by US	Mute
13	Boy	5/2016	35	36	24	Unknown	37	Pregnancy	27	-5.0	Died at 2 years	NA	Microcephaly and myelomeningocele (Suppl. Fig 7 a and b), fetal growth retardation and polyhydramnios by US	Seizures, multiple arthrogryposis, microcephaly in death certificate
16	Boy	11/2016	14	24	30	First	35	Pregnancy	28	-2.9	Died at 47 days	NA	Microcephaly and clubfoot by US;	Multiple arthrogryposis, CT documented

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													Ventriculomegaly with dangling choroid plexus, encephalic calcifications, and abnormal slits in the brain by US; periventricular calcifications; ventriculomegaly, y with periventricular calcifications; thinning of sulci and gyri by CT	microcephaly, craniosynostosis, CMV (-), Toxoplasma (-), Rubella (-)
17	Girl	1/2017	-	-	23	First	39	Pregnancy	30	-2.8	Alive	NA	Microcephaly by TFUS	Clubfoot.
18	Boy	5/2016	18	19	31	Unknown	38	Pregnancy	25	-5.4	Stillbirth	NA	Microcephaly with Occipital encephalocele and overlapping cranial sutures and bell-shaped chest and polycystic kidney by US	--
19	Girl	9/2016	6	35	23	Unknown	37	Pregnancy	32	0.5	Alive	Positive	Strawberry shaped head, microcephaly, ventriculomegaly, hydrocephalus (Suppl Fig 8 a - c), hypogenesis of corpus callosum, obliteration of cisterna magna (Supp. Fig. 8 d) sacral meningocele (Suppl. Fig. 8 e), by US	Clubfoot, hip dysplasia. HSV IgG (+) IgM (-), Chikungunya (-) DENV NS1 (-)

US=ultrasound; TFUS=transfontanellar ultrasound; CsF=cerebrospinal fluid; MRI=magnetic resonance imaging; CT=computer tomography scan, NA=not available

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Annex 2

Table 2. Early development of 9 of the 13 children with probable congenital Zika syndrome, Tuxtla Gutierrez, Chiapas, 2019 7

ID	Z-score of Head Circumference	Age at follow-up |evaluation	Movement/Physical (months normal)						Language/Cognitive (months normal)					
			Held head up w/o support (4)	Rolls over (6)	Seats with support (5)	Seats without support (6)	Crawls (9)	Stands (9)	Turns head toward sound (2)	Begins to smile at people (2)	Follow things with eyes and recognize people at a distance (2)	Babbling (4)	Says 'mama' and 'papa' (9)	Tries to say words spoken (12)
1	-2.9	Deceased 2 mos.	NA						1	NA	NA			
4	-3.2	Deceased 7 mos.	4	6	6				1	4	7	5	6	
6	-3.6	Alive 32 mos.	4	18	12	30	Not then	12	6 - 8	2 - 3	2 - 3	Not then	24	Not then
7	-3.6	Alive 31 mos.	Was Not Evaluated											
8	-3.8	Deceased 12 mos.	Did not	Did not	Did not	Did not	Did not	Did not	4	7	2	Did not	7-8	Did not
9	-3.3	Alive 29 mos.				Not then		Not then				4	9	Did not
11	-4.6	Alive 24 mos.	Not then	12	Not then	Not then	Not then	Not then	1	4	9 - 10	4	Not then	Not then
12	-4.8	Alive 20 mos.				9		9	Not then				Not then	Not then
13	-5.0	Deceased 26 mos.					Did not							
16	-2.9	Deceased 1 mo.												
17	-2.8	Alive 26 mos.	5	14	11	12	12	Not then	NA	NA	NA	10	9	NA
18	-5.4	Stillbirth	Was not Evaluated											
19	0.5*	Alive 30 mos.						Not then						

*Had hydrocephalus

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Annex 3

Table 3. Characteristics of cases of probable congenital Zika syndrome and their controls, Hospital Pascacio Gamboa, 2017-2019

Characteristics		Cases	(%)	Controls	(%)	OR (95% CI)
Zika antibody titer by $PRNT_{80}$						
	Present	13	100.0	31	88.6	2.1 (0.3, ∞)
	Absent	0		4		1
	Total	13		35		*P*-value = 0.4
Fetal sex						
	Female	9	69.2	20	57.1	1.7 (0.4, 6.3)
	Male	4		15		1
	Total	13		35		*P*-value = 0.5
Maternal age						
	21+	11	84.6	20	57.1	4.4 (0.9, 22.9)
	16-20	2		15		1
	Total	13		35		*P*-value = 0.1
Maternal education						
	<9 grade	12	92.3	25	65.7	6.2 (0.7, 53.4)
	10+ grade	1		13		1
	Total	13		35		*P*-value = 0.08
Periconceptional folic acid supplementation						
	Yes	2	15.4	3	8.6	1.9 (0.3, 13.2)
	No	11		32		1
	Total	13		35		*P*-value = 0.6
Use water containers						
	Yes	13	100.0	33	86.8	2.5 (0.4, ∞)
	No	0		5		1
	Total	13		35		*P*-value = 0.3
Regular bed net use						
	Yes	6	46.2	20	57.1	0.7 (0.2, 2.5)
	No	7		15		1
	Total	13		35		*P*-value = 0.7

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Annex 4

Table 4. Characteristics of cases of probable congenital Zika syndrome and their controls, Hospital Pascacio Gamboa, 2017-2019

Titers	Cases	(95% CI)	Controls	(95% CI)	t-test *P*-value
DENV1 Geometric mean (log_{10})	548.7	(234.3, 1285.0)	363.2	(219.1, 603.7)	0.38
DENV2 Geometric mean (log_{10})	119.0	(30.0, 471.6)	161.7	(75.1, 348.4)	0.68
DENV1 >1:80 or DENV2 >1:80 $PRNT_{80}$ Ab	Cases	(%)	Controls	(5)	Exact odds ratio (95% CI)
Present	12	92.3	31	88.6	1.5 (0.1, 82.5)
Absent	1		4		1
Total	13		35		*P*-value = 1.0
DENV1 $PRNT_{80}$ Ab	Cases	(%)	Controls	(%)	Exact odds ratio (95% CI)
640+	10	76.9	21	60.0	2.2 (0.5, 14.6)
<640	3		14		1
Total	13		35		*P*-value = 0.2
Ratio of DENV1 to DENV2 $PRNT_{80}$ Ab	Cases	(%)	Controls	(%)	Exact odds ratio (95% CI)
≥10	5	38.5	5	14.3	3.6 (0.7, 20.5)
<10	8		30		1
Total	13		35		*P*-value=0.08
Ratio of DENV1 to DENV2 ≥10$PRNT_{80}$ Ab Adjusted for					Exact odds ratio (95% CI)
Maternal age					2.7 (0.5, 15.8)
Maternal education					2.9 (0.5, 16.71)
Periconceptional folic acid supplementation					3.6 (0.7, 20.5)
Use water containers					3.1(0.8, 18.0)
Bed net use					3.3 (0.6, 19.8)
Use of water containers and bed net use					5.5 (0.7, 70.8)

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