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Supplementary appendix

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Supplement to: França GVA, Schuler-Faccini L, Oliveira WK, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. *Lancet* 2016; published online June 29. http://dx.doi.org/10.1016/S0140-6736(16)30902-3.

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Supplementary appendix

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Supplement to: França GVA, et al. Congenital Zika virus syndrome in Brazil: case series with the first 1,501 live births with complete investigation.

Contents

Description of the surveillance system

Step 1. Local hospitals or health centers report to the Municipal Secretariat of Health (a) live born infants with microcephaly, and (b) fetuses, miscarriages or stillbirths with suggestive imaging or laboratory findings. These constitute the **suspected cases**. Criteria for reporting live born infants are shown in Table A.

Step 2. The Municipality Secretariat of Health enters the information on suspected cases into the National Public Health Events Database (RESP or *Registro de Eventos de Saúde Pública*)[1] of the Ministry of Health.[1] Such cases are immediately notified to the State Surveillance System corresponding to the municipality where the case was reported.

Step 3. After the suspected case is reported, the State Surveillance System, in partnership with the municipal health services, launches an investigation aimed at classifying each case as "confirmed" or "discarded" for microcephaly due to congenital infections. Figure 1 in the main article shows a flowchart that guides this process. Because some cases required imaging or specialized clinical assessments that are not available in most municipalities, this process can take several weeks.[2] MOH guidelines (Table B) are used for imaging results.

Step 4. As the State concludes the investigation of each suspected cases, the MOH is informed whether the case was confirmed or discarded, and this information is entered in the national database.

[1] The only exception is the State of Pernambuco, which has its own separate database. For the present analyses, this database was merged with the national database so as to include all suspected cases reported in the country.

[2] Of 3,174 cases reported up to Jan 2, 2016, 32.5% had been deemed as fully investigated by Feb 27, 2016; and of 1,609 cases reported during January, only 20.5% had been fully investigated by Feb 27.

Table A. Microcephaly case definitions for live born infants adopted by the Ministry of Health (MOH) over time

Period	Cutoff for term newborns	Cutoff for preterm newborns
17 Nov to 12 Dec 2015	<=33 cm for both sexes	≤3rd centile of the Fenton reference[2] by gestational age and sex
12 Dec 2015 to 12 Mar 2016	<=32 cm for both sexes	≤3rd centile of the Fenton reference[2] by gestational age and sex
13 March 2016 to the present*	<- 2 SD (WHO Standards) for term (<31·5 cm for girls and 31·9 for boys)	<-2 SD of Intergrowth[3] reference by gestational age and sex

Source: Brasil. Ministério da Saúde. [4]

(*) The last change in criteria took place after the present case series was accrued.

Panel A. TORCH (toxoplasmosis, other, rubella, cytomegalovirus and herpes) infections

TORCH in the MOH surveillance system

When the surveillance system was set up in November 2015, States were asked to rule out TORCH congenital infections before classifying a suspected case as "confirmed". It soon became evident that the requirement of laboratory investigation for TORCH (particularly testing for cytomegalovirus) was leading to a large backlog of cases under investigation. In January 2016 the MOH changed the focus of the surveillance system from ZIKV to all congenital infections and no longer required ruling out TORCH. Thus, "confirmed cases" currently in the system also include some cases due to other infections.

When TORCH test results were available, these were reported to the MOH. In the present re-analyses of suspected cases, we attempted to rule out such infections (i.e., the "highly probable" category in our analyses excludes syphilis, toxoplasmosis and cytomegalovirus).

Epidemiology of TORCH in Brazil

We reviewed the frequency of TORCH congenital infections in Brazil to decide how to handle these infections when classifying suspected cases.

We did not locate any population-based studies on the frequency of any of the TORCH congenital infections in Brazil. The MOH states that **rubella** has been eradicated, with the last case of congenital rubella syndrome reported in June 2009.[5] Our database included three cases with positive IgM for rubella, which may represent false positives, and were not taken into account in the classification. Regarding **toxoplasmosis**, a review of local studies found a prevalence range from 0·5 to 2·3 per 1,000;[6] these included a few reports of microcephaly in affected newborns. Data on **cytomegalovirus** congenital infection are even less available: a large screening study of volunteer women showed 1 per 1,000 newborns[7], and among newborns in intensive care the prevalence was 8 per 1,000.[8] Cytomegalovirus is associated with microcephaly, ventricular enlargement and brain calcifications, but the majority of children born with congenital CMV are asymptomatic during the neonatal period and would not have been reported.[9] Congenital **syphilis** is frequently notified to the MOH – over 1,600 cases in the Northeast in 2013. However, syphilis rarely causes microcephaly or brain calcifications.[10] Finally, although congenital **herpes virus** infection is associated with microcephaly, this seems to be a rare condition[11] because 80 to 90% of transmission occurs during delivery.[12,13]

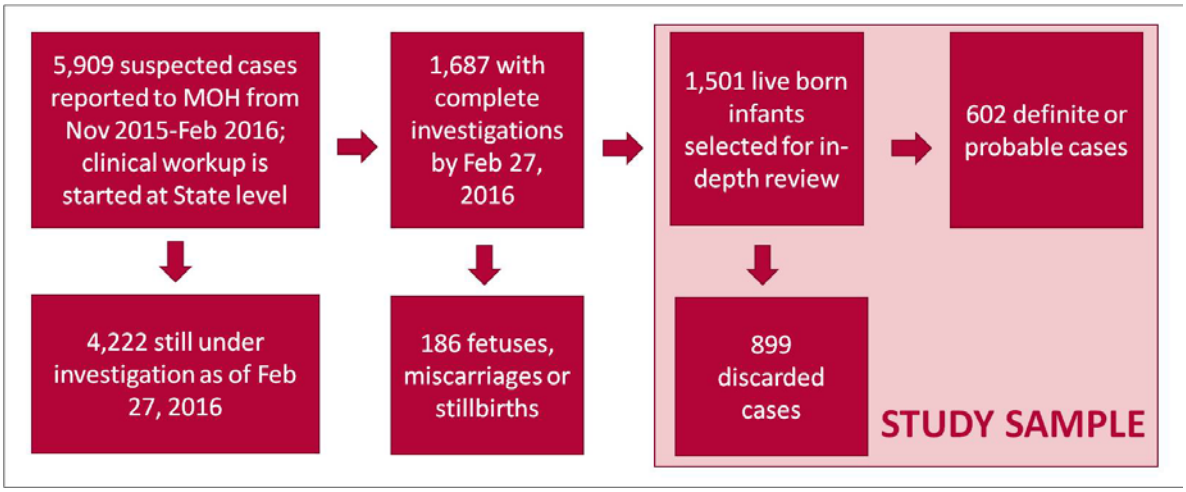
Because few of our suspected cases were tested for herpes or rubella, we prioritized laboratory findings on syphilis, toxoplasmosis and cytomegalovirus to rule out other congenital infections in our classification of highly probable cases.

Table B. Main central nervous system alterations suggestive of congenital infection observed through imaging methods, according to MOH guidelines.

Examination	Findings
Ultrasound during pregnancy	• Calcifications, or • Ventricular enlargement, or • At least two of the following posterior fossa brain malformations: o hypoplasia of the cerebellar vermis o enlargement of the posterior fossa greater than 10 mm o agenesis / hypoplasia of the corpus callosum
Transfontanellar ultrasound	• Diffuse cortical atrophy • Encephalomalacia • Calcifications • Ex vacuo ventriculomegaly • Dysgenesis of the corpus callosum • Atrophy of the corpus callosum • Cerebellar atrophy with thickening of the tentorium
Computerized tomography (CT) and Magnetic Resonance Imaging (MRI)	• Calcifications in the brain parenchyma • Ventriculomegaly • Malformation of cortical development • Abnormal gyration pattern (lissencephaly, pachygyria, polymicrogyria) • Hypoplasia of the brainstem and cerebellum • Decreased white matter attenuation

Source: Brasil. Ministério da Saúde. [4]

Figure A. Flowchart showing how the study sample of 1,501 suspected cases in live born infants relate to the total number of cases reported up to February 27, 2016



Source: Brasil. Ministério da Saúde. [4]

Panel B. Missing data for suspected cases

Table C shows data availability for the five categories of cases.

Information on region of the country and sex were available for virtually all cases. Data on gestational age was (slightly) more frequently available for discarded than for definite/probable cases.

Head circumference measures were available for 1,391 (92·7%) of all suspected cases, and Z-scores could be estimated for 1,258 (83·8%) cases that also had values of gestational age at birth. Missing values for Z-scores were similarly frequent for both sexes (15·5% for boys and 16·4% for girls; P=0·35) but were unavailable for 12·8% (77/602) of definite/probable cases, and 6·8% (61/899) of discarded cases (P<0·001) (Table C).

Reported head circumferences showed strong digit preference, with 85·6% (1,191/1,391) of all measurements being in full cm, and 13·1% (182/1,391) being rounded to 5 mm. Similarly, 98·6% (1,344/1,363) of gestational ages were expressed in full weeks.

Information on rash was not collected routinely in some states, and therefore missing values were common, particularly for discarded cases. Likewise, mortality data were available for almost all definite/probable cases but missing for 20% of discarded cases.

Detailed imaging reports were available for 686 suspected cases (ultrasound results for were available for 608, computerized tomography for 121 and magnetic resonance for 15 newborns); 563 were not subjected to imaging examinations (table C) and for the remaining 252 results were mentioned by the type of exam was not specified.

By definition, all cases in the highly and moderately probable categories had detailed results. Among these, 40·9% (70/171) presented only calcifications, 21·1% (36/171) only ventricular enlargement, and 38·0% (65/171) presented both. The latter group accounted for 37·0% (20/54) of the cases in the highly probable and 24·9% (45/181) of those in the moderately probable categories.

Laboratory information on ZIKV was only available for 90 cases; the 76 with positive results (67 serology and nine PCR) were classified as definite cases. Initially, few laboratories in the country offered testing for ZIKV, and although this number is expanding rapidly, this was an important limitation at the time the case series was investigated.

Laboratory results for syphilis, toxoplasmosis or cytomegalovirus were available for all highly probable cases – as negative results were a criterion for inclusion in this group - but 472 moderately or somewhat probable cases did not have results for all three infections.

Table C. Percent of cases with available data, and distribution of imaging and laboratory results by group.

		Definite	Highly probable	Moderately probable	Somewhat probable	Discarded	P level[a]
Region of the country		100.0	100.0	100.0	100.0	100.0	1.00
Sex		98.7	100.0	100.0	99.0	99.8	0.19
Gestational age		94.7	96.3	97.2	96.9	99.0	0.02
Head circumference		89.5	77.8	89.0	87.3	93.2	<0.001
Presence of a rash		9.2	88.9	72.9	44.3	38.7	<0.001
Mortality		96.1	92.6	84.5	92.8	78.3	<0.001
Imaging details	Specific findings described	18.4	100.0	100.0	0.0	2.2[b]	<0.001
	Exam described as abnormal without details	0.0	0.0	0.0	100.0	0.4[b]	
	Exam described as normal	5.3[c]	0.0	0.0	0.0	40.8	
	No results provided	76.3[c]	0.0	0.0	0.0	56.2[e]	
ZIKV laboratory results		100.0	5.6[d]	0.0	0.3[d]	1.1	<0.001
STC laboratory results	All 3 STC infections reported	2.6	100.0	0.0	9.3	9.2	<0.001
	1-2 STC infections reported	11.8	0.0	36.5	16.8	33.6	
	No results provided	80.3	0.0	63.5	73.9	52.1	
Number of cases		76	54	181	291	899	

(a) Chi-squared test for comparison of the five categories of cases

(b) Cases with suggestive imaging findings but who also had positive results for a TORCH infection.

(c) Cases with positive ZIKV laboratory results that were considered as "definite cases" in spite of the imaging results.

(d) Cases with negative PCR for ZIKV; because of the short time period when PCR remains positive, ZIKV was not ruled out.

(e) Cases who were discarded after clinical examination, or for whom the State did not report on imaging findings.

Table D. Comparison of cases that were discarded due to clinical/anthropometric criteria or due to imaging/laboratory criteria

This table is based on the criteria used by the states and municipalities to discard suspected cases, according to which 901 cases were dismissed; the revised classification presented in this paper refer to 899 discarded cases.

Figure A shows that suspected cases could be discarded due to two groups of reasons:

- Image/laboratory: cases had normal results in imaging examinations or had negative serology for ZIKV.
- Clinical/anthropometric: includes suspected cases that had been wrongly classified in terms of anthropometry (e.g. incorrect measurement of head circumference, inaccurate gestational age estimation, or being above the cutoff point), or that were dismissed on clinical grounds (e.g. did not present craniofacial disproportion, presented other causes associated with microcephaly (preterm birth or low birthweight) and had normal neuro-psychomotor development).

Tables D1 and D2 show that suspected cases discarded due to imaging/laboratory criteria included a higher proportion of newborns from the Northeast region and whose mothers had reported a rash, and had slightly smaller head circumference Z scores. Both groups were similar in terms of sex, gestational age, and birthweight. Differences in mortality tended to go in the opposite direction but the number of deaths was small (P=0·087).

These results are compatible with higher-risk newborns being more likely to be subjected to imaging/laboratory testing, but the differences between the two groups tended to be small.

Table D1. Comparison of the two groups of discarded cases according to region, sex, gestational age, reported rash and mortality by the median age of 8 days.

	Image/laboratory		Clinical/anthropometric		P-value*
	n	%	N	%	
Northeast region*	318	89·8	426	77·9	<0·001
Female sex*	229	64·7	352	64·6	1·00
Gestational age*					
<37	23	6·5	37	6·9	0·127
37-38	110	31·2	202	37·5	0·001
>=39	220	62·3	300	55·7	
Reported rash	47	23·7	14	10·1	
Deaths	1	0·3	8	2·0	0·087
Total	354		547		

*Fisher's exact

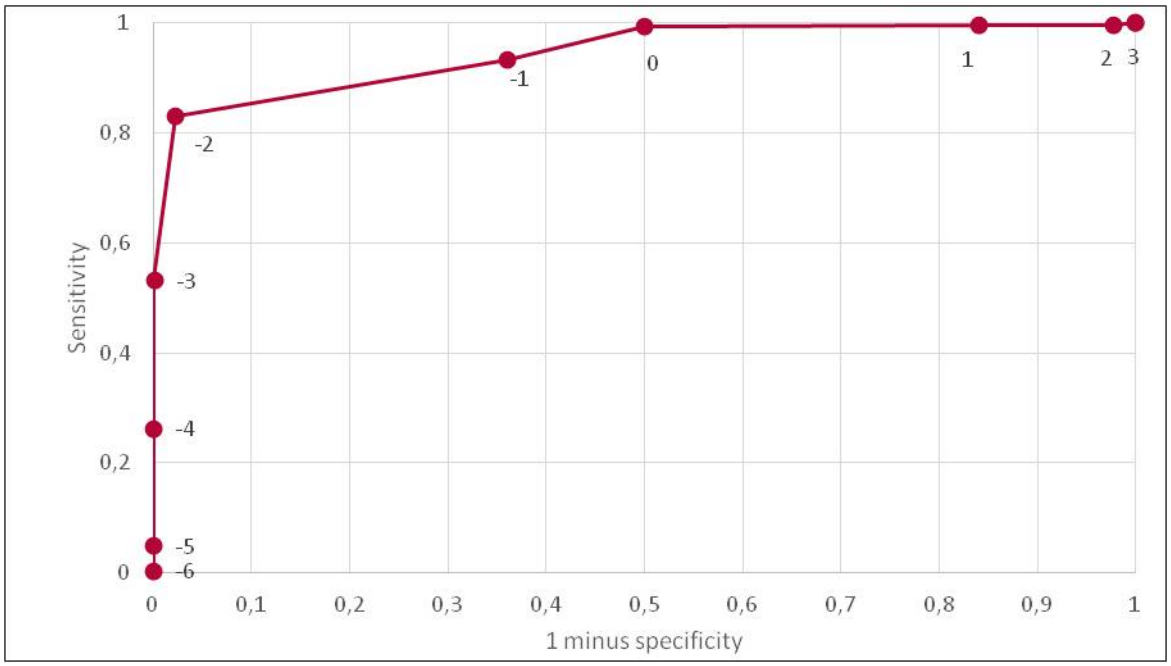
Table D2. Comparison of the two groups of discarded cases according to anthropometric characteristics.

	Laboratory/image			Clinical/anthropometric			P-value
	n	Mean	SD	n	Mean	SD	
Male							
Head circumference (cm)	120	31·84	0·09	179	31·93	0·10	0·3678**
Head circumference Z-scores	119	-1·78	0·08	177	-1·52	0·07	0·0190*
Birthweight (g)	121	2758·34	43·02	184	2769·22	36·43	0·8484*
Female							
Head circumference (cm)	220	31·74	0·07	333	31·90	0·08	0·1738**
Head circumference Z-scores	219	-1·52	0·06	327	-1·36	0·06	0·0626**
Birthweight (g)	222	2768·44	28·57	331	2767·05	22·32	0·9691*

*t-test

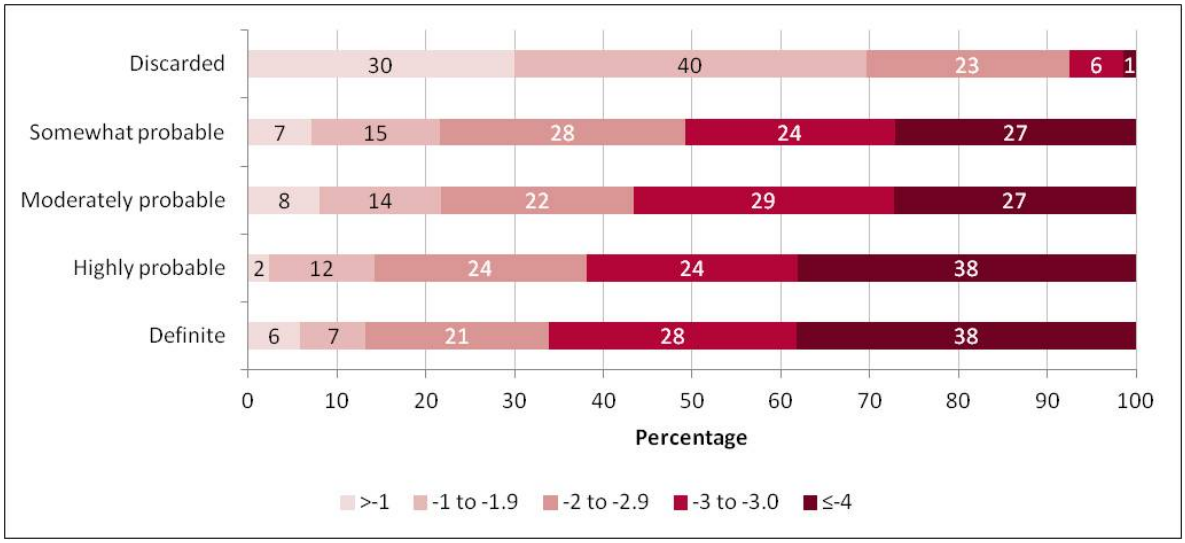
**Wilcoxon rank-sum (Mann-Whitney) test

Figure B. Receiver operating characteristic (ROC) curve for definite/probable congenital Zika virus syndrome.



Note: Sensitivity is based on the 602 definite/probable cases, and specificity on the normal distribution of head circumferences (InterGrowth reference for preterm and term newborns). The numbers in the figure represent cut-off points expressed as Z-scores.

Figure C. Distribution of the five categories of suspected cases according to head circumference Z-scores by gestational age and sex.



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