REVIEW

Imaging of congenital Zika virus infection: the route to identification of prognostic factors

Manon Vouga[1] and David Baud[2]*

[1]Materno-fetal and Obstetrics Research Unit, Department of Obstetrics and Gynecology, Maternity, University Hospital, Lausanne, Switzerland
[2]Institute of Microbiology, Faculty of Biology and Medicine, University of Lausanne and University Hospital, Lausanne, Switzerland
*Correspondence to: David Baud. E-mail: david.baud@chuv.ch

ABSTRACT

Zika virus (ZIKV) has recently emerged as a novel teratogenic agent associated with severe neurological complications. The risk associated with maternal infection remains to be exactly defined but appears to be significant. Like other TORCH agents (toxoplasmosis, other agents, rubella, cytomegalovirus and herpes simplex), it is unlikely that all affected fetuses will be symptomatic at birth. It is therefore urgent to better define the spectrum of anomalies observed in infected fetuses to provide adequate parental counseling. In this review, we provide a comprehensive analysis of major cases described to date and highlight specific prenatal and postnatal radiological findings of congenital ZIKV infection. A total of 19 reports were included in our analysis. ZIKV seemed to harbor a specific tropism for the central nervous system, and anomalies were mostly limited to the brain. Major radiological findings were ventriculomegaly, diffuse calcifications and signs of abnormal gyration as well as cortical development. In addition, a significant number of fetuses suffered from intra uterine growth restriction. Based on these findings, we provide recommendations for adequate radiological monitoring of at-risk pregnancies. © 2016 John Wiley & Sons, Ltd.

Funding sources: David Baud is supported by the 'Fondation Leenaards' through the 'Bourse pour la relève académique', the Divesa Foundation and the Swiss National Science Foundation (SNSF n310030-156169). Manon Vouga is funded through the MD-PhD grant by the Swiss National Science Foundation (SNSF) (no. 323530_158123).
Conflicts of interest: None declared

INTRODUCTION

The year 2015 has seen the emergence of a novel teratogenic infectious agent, Zika virus (ZIKV). A dramatic discovery of this nature has not been made since 1942, when first reports on congenital rubella syndrome occurred.[1] By the end of 2015, the Brazilian government noticed an increase in the incidence of microcephaly suspected to be related to the ongoing ZIKV epidemic.[2]

ZIKV is a member of the *Flaviridea* family, which also includes dengue and West Nile viruses. As these viruses are mostly transmitted through arthropods, they are also known as arboviruses (i.e. arthropods borne viruses).[3] Additional modes of transmission of ZIKV include blood products,[4] sexual intercourse[5] and, potentially, breastfeeding[6] and saliva,[7,8] although no cases have been confirmed so far. First isolated in 1947 in Uganda,[9] ZIKV had always been considered a benign infection; until 2007, only 14 human cases were described in the literature.[10] ZIKV infection is asymptomatic in 80% of cases. When reported, symptoms are mostly unspecific and mimic other viral diseases (pruritic maculo-papular rash associated with low-grade fever, asthenia, arthralgia/myalgia and conjunctivitis).[10] It is only with the recent outbreaks, first in French Polynesia and New Caledonia, and recently in the Americas, that severe-related neurological complications, in particular Guillain–Barre syndrome, and congenital malformations have emerged.[10,11] The small number of cases in previous epidemics as well as the lack of interest from developed countries may explain why these dramatic complications were not described earlier. Evidence has now accumulated and scientific communities agree that ZIKV should be considered similarly to the toxoplasmosis, other agents (e.g. Syphillis, varicella virus, herpes virus), rubella, cytomegalovirus and herpes simplex (TORCH) agents.[12] Figure 1 highlights the major events in the history of ZIKV leading to its acceptance as an emerging teratogenic agent.

Nevertheless, many questions remain to be answered.[13] The magnitude of the epidemic, especially affecting countries with high pregnancy rates, suggests that even in the case of a low-transmission risk, the number of potential infected fetuses could be significant. Currently, over 2 billion people are living in areas with potential ZIKV circulation, representing 5.42 million potential pregnancy exposures in 2015.[14] Serosurveys from previous epidemics in Yap Island and French Polynesia, in which 50–72.5% of the population were infected,[10] suggest

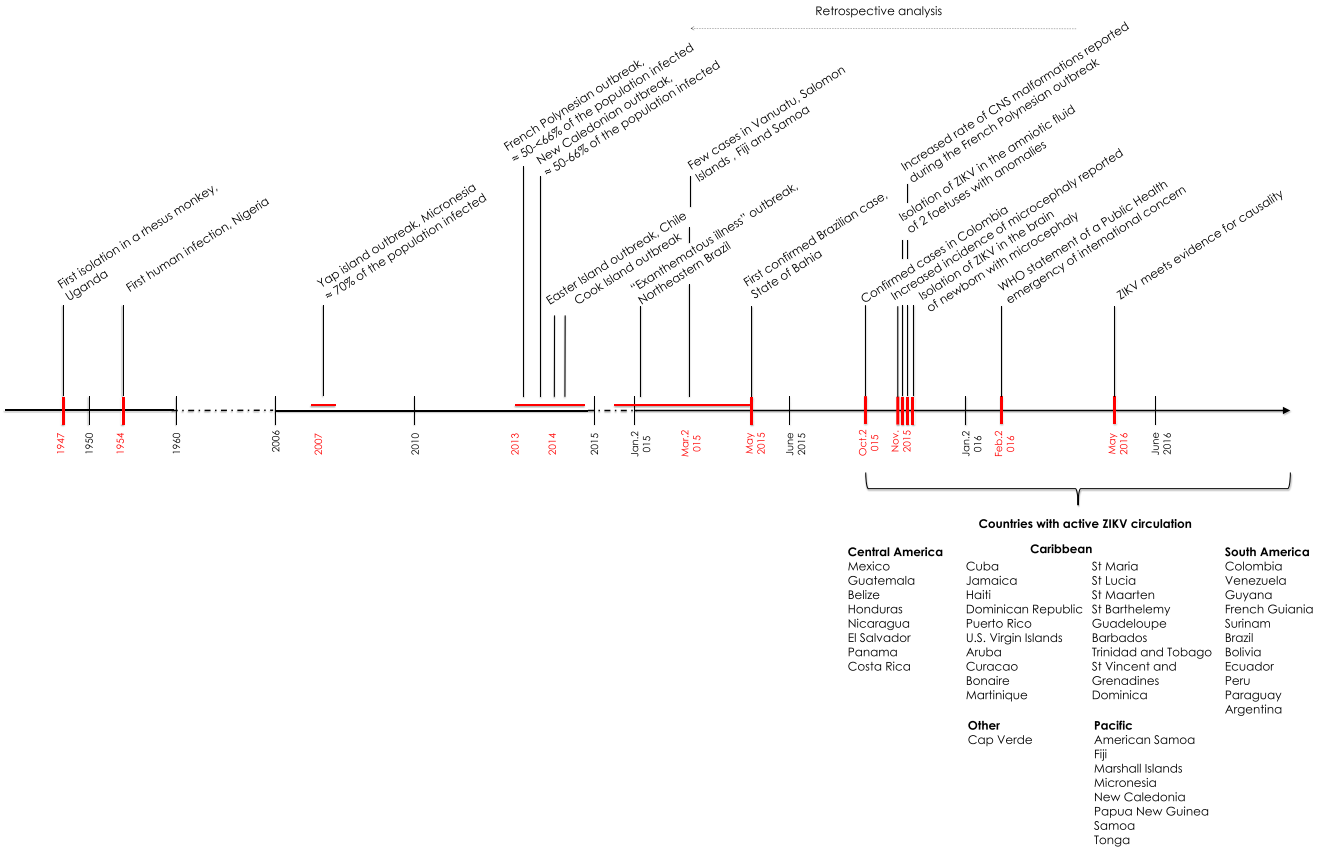

Figure 1 History of Zika virus (ZIKV). This timeline presents the most important events in ZIKV history that have lead to its emergence as a teratogenic agent. In addition, it presents a list of countries with active circulation, as to date on June 16, according to the American Centers of Disease Control[58]: 1947,[9,10] 1954,[10,78] 2007,[10,79] 2014,[10,80] January–March 2015,[10,81] November 2015,[20,21,82–84] February 2016[85] and May 2016[12]

a high rate of human infection in case of epidemic in a non-immune population, as observed today.

In that context, it is critical to rapidly characterize the spectrum of anomalies and prognosis of infected fetuses to provide adequate parental counseling. Similarly to other TORCH agents, it is likely that not all infected fetuses will develop symptoms. The challenge will therefore be to identify fetuses with a poor prognosis. For other congenital infections, the presence of fetal anomalies, particularly of the brain, is considered the most specific predictive factor of severe symptoms at birth[15,16]; it is accepted as justification for termination of pregnancy (TOP) by most obstetrical associations.[17,18] It might be speculated that a similar course exists for ZIKV-infected fetuses. It is therefore important to better understand the spectrum of anomalies observed in ZIKV-infected fetuses and their prognosis. We thus provide a comprehensive analysis of major cases described so far and highlight specific prenatal and postnatal radiological findings of congenital ZIKV infection. In light of these findings, we provide recommendations for adequate radiological monitoring of at risk pregnancies.

METHOD

Using the search terms 'ZIKA' and 'pregnancy', we systematically searched PubMed for published studies (Appendix 1). Alternative spellings were used for search terms with multiple accepted spellings (ZIKV, pregnant). To ensure completeness, the references of extracted articles and review articles were also analyzed. The two authors evaluated the articles and extracted data. Searches were limited to English language. We excluded articles that were not based on 'fetus/fetal' or 'neonate/neonatal' or did not provide adequate imaging description.

PERINATAL TRANSMISSION OF ZIKA VIRUS

The first perinatal transmission was described by Besnard *et al.*[19] and likely occurred during delivery. Subsequently, Calvet *et al.*[20,21] isolated ZIKV in the amniotic fluid of two fetuses with significant cerebral malformations whose mothers had presented with symptoms compatible with a ZIKV infection, supporting a transplacental transmission of the virus. Transplacental transmission has now been confirmed by *in vivo* experimental studies.[22,23]

Although now commonly accepted, the risk of materno-fetal transmission as well as the gestational ages (GA) during which exposure carries the highest risk of malformations is currently unknown. So far, ZIKV infection during pregnancy has been associated with early and late miscarriages, stillbirths, intra uterine growth restriction (IUGR), hydrops fetalis and cerebral fetal malformations (recently reviewed by Panchaud *et al.*[11]).

The ZIKV congenital syndrome seems to include microcephaly associated with other cerebral malformations that may lead to severe mental retardation and significant motor disabilities, ocular anomalies and auditory defects.[11,24,25] Additional malformations have been occasionally observed, such as hypospadias, cryptorchidia and mircopenis.[26,27] Based on a retrospective analysis of epidemiological data from previous outbreaks in the Pacific as well preliminary data from Brazilian surveys, several groups have tried to provide risk estimations. These reports have evaluated the risk of microcephaly to be the highest in cases of maternal infection during the first trimester,[28–30] with an absolute risk ranging from approximately 1–14%, depending on the assumed rate of exposition in the population.[29,31] This correlates with the recent findings in a cohort of 1850 Columbian pregnant women, in which 612 contracted ZIKV in the third trimester and none of their newborns presented with microcephaly; results regarding outcomes from pregnant women exposed earlier in pregnancy are still pending.[32] Nevertheless, these reports are based on the assumption of exposure to ZIKV, and further studies are needed to confirm these estimations. Cerebral anomalies (ventriculomegaly and calcifications) have been observed in cases of suspected infections occurring as late as 27 weeks of gestation (WG).[33] Finally, even if the risk of severe cerebral anomalies may be lower in case of infections occurring in late second or third trimesters, other adverse pregnancy outcomes have been observed in particular IUGR and stillbirths.[33] Additional studies are needed to better characterize the spectrum of disease at each GA.

RADIOLOGICAL FINDINGS OF ZIKV CONGENITAL INFECTION

Prenatal findings

We isolated 14 publications, with a total of 66 cases, providing adequate prenatal imaging descriptions.[20,21,26,27,33–42] In addition to ultrasounds, six reports also provided information on fetal magnetic resonance imaging (MRI).[27,34,36,37,39–41] Major findings are summarized in Table 1. A time lapse of at least 3 weeks[20,21] and up to 15 weeks[35] was observed between suspected maternal infection and identification of fetal anomalies. The most frequent prenatal findings (>10% of reviewed cases) were a reduced head circumference (HC), ventriculomegaly, calcifications and neuronal migration anomalies, marked by lissencephaly, pachy/agyria, polymycrogyria or opercular anomalies. In addition, dysgenesis of the corpus callosum, either directly observed or suggested by the rupture of the septum pellucidum, was frequently observed, a finding also reported in toxoplasmosis[15] and cytomegalovirus (CMV) infections.[43]

Ventriculomegaly

Contrary to what is observed for toxoplasmosis or lymphocytic choriomeningitis virus (LCMV, another recently discovered teratogenic agent), in which ventriculomegaly is often bilateral and symmetrical,[44,45] ZIKV-induced ventriculomegaly most frequently demonstrated a non-hypertensive pattern and was often asymmetrical or unilateral. In toxoplasmosis or LCMV, ventriculomegaly is thought to be due to Sylvius's aqueduct obstruction by necrotizing process.[44,46] ZIKV-associated ventriculomegaly, however, is probably related to the cerebral atrophy, as suggested by the thinning of the cortical mantle frequently observed simultaneously.

Calcifications

Calcifications have been described to correspond to area of focal necrosis, often with poor calcification at the time of prenatal diagnosis and therefore appearing as echogenic foci without posterior shadowing effect.[15,43,44] In ZIKV-related cases, calcifications were mostly localized at the cortico-sub cortical white matter junction and did not seem to harbor the specific periventricular localization frequently observed in CMV or LCMV infections,[43,45] although periventricular calcifications were also sometimes observed. Additionally, calcifications were also observed in the midbrain, basal ganglia, brainstem and cerebellum similarly to what may be observed for toxoplasmosis, CMV and LCMV.[15,43,45] In addition to widespread calcifications, dysgenesis of the cerebellum, brainstem, thalamus, basal ganglia and spinal cord was reported in some cases, highlighting the diffuse lesions induced by ZIKV. Cerebellar hypoplasia has frequently been described in case of CMV[43] or LCMV.[45] For the latter, it has even been described as the sole abnormal findings in two cases.[45] Only one report described partial cerebellar hypoplasia in a case of toxoplasmosis,[47] but this may be due to the low number of reports including fetal MRI.

Microcephaly

A reduced HC < -2SD, suggestive of a microcephaly, was the third most common finding observed after ventriculomegaly and calcifications. Caution should be made, as authors did not always precise the cut-off used, and as discussed later, a cut-off of HC < -3SD is more appropriate.[48] Importantly, this finding was always found in association with other cerebral anomalies. In the report by Driggers *et al.*,[40] the HC remained within normal ranges, but a decrease from the 47th to the 24th percentile between the 16 and 20 WG, when pregnancy was terminated, was observed, suggesting that a pathological HC may have been reached later on. Congruently, in all reports, the diagnosis of microcephaly was made between 26 and 33 WG and suggests that it is probably a late finding. Others described normal HC despite other severe brain anomalies. In the study of Franca *et al.*[49] evaluating 1501 Brazilian newborns, one in five definite or probable cases of ZIKV infection presented an HC within normal range at birth.

Destruction of the germinal matrix, abnormal migration and cortical organization

Abnormal migration and cortical organization are shown by abnormal gyration that is reduced (pachy/agyria) or increased (polymycrogyria), heterotopias and dysgenesis of the corpus callosum. In addition, destructive cystic lesions such as porencephaly, schizencephaly or in extreme forms, hydranencephaly, are frequently observed because of previous

Table 1 **Prenatal finding associated with ZIKV-suspected congenital infection**

	Total number of patients n (%)	French Polynesian Cohort [27,36,37]	Calvet *et al.*, Oliveira Melo *et al.* [20,21]	Mlakar *et al.* [35]	Sarno *et al.* [38]	Werner *et al.* [39]	Moron *et al.* [34]	Driggers *et al.* [40]	Brasil *et al.* [33]	Culjat *et al.* [26]	Werner *et al.* [41]	Perez *et al.* [42]
Number of patients / study	**66**	14	2	1	1	1	1	1	42	1	1	1
Imaging		US/MRI	US	US	US	US/MRI	US/MRI		US	US	US/MRI	US
ZIKV fetus' status		4 confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed
Fetal vitality												
Stillbirth	3 (5)				1				2			
Abnormal fetal movements	4 (6)	3		1								
Fetal biometry												
IUGR	9 (14)	1		1	1				5		1	
Fetal tonus												
Arthrogyroposis	2 (3)								1			1
Amniotic fluid												
Polyhydramnios	0 (0)											
Oligoamnios	2 (3)								2			
Placenta	**8 (12)**											
Abnormal thickness	3 (5)						1		2			
Calcifications	5 (8)	4		1			1					
Doppler	**4 (6)**		normal	normal			normal		4			
Abnormal MCA flow	3 (5)								3			
Abnormal umbilical doppler	1								1			
Cerebral	**30 (45)**											
General												
Microcephaly	15-17 (24)	8	2	1	1	1	1		2[a]		1	
<3 SD			1			1	1					
Enlargement of the subarachnoid space	11 (17)	5	1					1	4			
Lissencephaly	2 (3)					1	1				1	
Hydranencephaly	1 (1)				1							
Cortex												
Calcifications	17 (27)	6	1	1	1	1	1		5	1	1	[a]
Abnormal gyration	7 (11)	5				1	1				1	
Opercular dysplasia	6 (9)	6										

(*Continues*)

Table 1 **(Continued)**

	Total number of patients n (%)	French Polynesian Cohort [27,36,37]	Calvet *et al.* Oliveira Melo *et al.* [20,21]	Mlakar *et al.* [35]	Sarno *et al.* [38]	Werner *et al.* [39]	Moron *et al.* [34]	Driggers *et al.* [40]	Brasil *et al.* [33]	Culjat *et al.* [26]	Werner *et al.* [41]	Perez *et al.* [42]
Diminution of cerebral mantle/ Cortical atrophy	3 (5)						1	1		1		
Corpus callosum												
Digenesis/ aplasia / hypoplasia	11 (17)	7	2			1		1			1	
Absence/rupture of septum pellucidum	10 (16)	9						1				
Ventricles												
Ventriculomegaly	21 (33)	13	1	1		1	1	1	2	1	1	1
Hypertrophy/cyst of the choroid plexus	2 (3)							1	1			
Ependymal pseudocyst	3 (5)	3										
Blake pouch cyst	1 (1)								1			
Cerebellum												
Hypoplasia/ Atrophia	1 (1)								1			
Vermis dysgenesis aplasia / hypotrophia	4 (6)	3	1									
Calcifications	2 (3)		1						1			
Basal ganglia												
Calcifications	1 (1)		1									
Dysgenesis	1 (1)		1									
Thalamus												
Dysgenesis/atrophia	1 (1)		1									
Brainstem												
Hypotrophia	1 (1)		1									
Ocular	**1** (1)											
Microphtalmy	1 (1)		1									
Cataracts	1 (1)		1									

(*Continues*)

Table 1 **(Continued)**

	Total number of patients n (%)	French Polynesian Cohort [27,36,37]	Calvet *et al.*, Oliveira Melo *et al.* [20,21]	Mlakar *et al.* [35]	Sarno *et al.* [38]	Werner *et al.* [39]	Moron *et al.* [34]	Driggers *et al.* [40]	Brasil *et al.* [33]	Culjat *et al.* [26]	Werner *et al.* [41]	Perez *et al.* [42]
Calcifications	1 (1)		1									
Others	**11 (17)**											
Anasarca/hydrops fetalis	1 (1)				1							
Clubfoot	1 (1)								1			
Micropenis	1-2 (3)	1-2										
Intestinal hyperechogeneicity	1 (1)	1										
Redundant scalp skin	1 (1)						1					
Liver anomalies	1 (1)								1			
Laparoschisis	1 (1)	1										
Multiple pterygium	2 (3)	1										1[b]
Rachischisis	2 (3)	2										
Facial dysmorphia	1 (1)	1									1	

This table presents the major findings of 14 studies presenting adequate radiological findings of suspected or confirmed ZIKV-infected fetuses. The French Polynesian cohort groups three retrospective studies, all analyzing the same cases of newborns born with significant neurological anomalies and/or microcephaly. Five cases presented in these reports showed only brainstem dysfunction without other anomalies; maternal serology for ZIKV was negative for all cases, and none of the mothers presented symptoms compatible with a ZIKV infection during pregnancy. Therefore, these five cases were excluded from our analysis. Out of the remaining 14 cases, it was possible to perform a retrospective ZIKV RT-PCR in the amniotic fluid of seven cases, among which four were positive. Among all patients, seven were evaluated through both MRI and US, and seven only through US. The reports by Melo *et al.*[20] and Calvet *et al.*[21] were also analyzed together as they report the same two cases. Some fetuses presented isolated anomalies: two cases of fetal demise noted on the 36 and 38 WG ultrasound, respectively, one case of abnormal middle cerebral artery flow, one case of isolated anhydramnios and one case of isolated IUGR with an abnormal umbilical Doppler.[33] Regarding cerebral anomalies, no isolated anomalies were identified.

Abnormal gyration refers to polymicrogyria and/or agyria and/or pachygyria.

ZIKV, Zika virus; IUGR, intra uterine growth restriction; MIR, magnetic resonance imaging; SD, standard deviation.

[a]Calcifications were noted on postmortem histopathological analysis, though not reported in the prenatal US performed at 19 WG.

[b]Fibrous proliferation in interarticularis spaces was noted on postmortem evaluation at 21 WG.

necrosis of neural progenitors. Similar destructive lesions are frequently observed in other congenital infections[15,43,45] but were not frequently reported in ZIKV cases, with hydranencephaly only observed in one case.[38]

Interestingly, no ventricular hemorrhages, indirectly reflecting a direct damage to the highly vascularized germinal matrix (GM), were observed in the reports analyzed here. Although suspected in one case on prenatal ultrasound, ventricular hemorrhage was not confirmed by fetal MRI.[40] Damage to the GM, however, was indirectly shown by the identification of occipital sub-ependymal pseudocysts.[27] Sub-ependymal pseudocysts are thought to develop following GM hemorrhages and are frequently observed in premature newborns[50]; alternatively, intraventricular synechia may also develop but have not been highlighted in the cases reported here. Although most of these pseudocysts are benign, when located in the occipital or temporal horn, they are associated with poor outcomes, due to early destruction of the GM.[51] Destruction of the GM and corresponding anomalies are also frequently observed in CMV or LCMV infections[43,45,46] but are less common in case of toxoplasmosis infections.[15,44]

Additional findings

Signs of placental inflammation such as increased thickness and calcifications were observed in some cases. Such placental anomalies have also been described in toxoplasmosis and are unspecific.[44] Placental dysfunction induced by ZIKV infection has been suggested to contribute to the development of brain damage, especially in case of early infection, when maternal placental circulation is not yet established.[52] The identification of sonographic markers of placental inflammation may support this hypothesis.

Lastly, IUGR was observed in 14% of ZIKV cases and could be related to both the direct effect of fetal infection and/or placental insufficiency. Additional findings associated with placental insufficiency (i.e. abnormal Doppler studies or oligoamnios), however, were only reported in three cases.[21] Nevertheless, this appears to be an important finding, as an *in vivo* model-confirmed IUGR induced by ZIKV infection.[23]

Postnatal findings

We identified nine studies, with a total of 158 cases, providing an adequate description of postnatal imaging of children born either with microcephaly suspected to be related to ZIKV[2,27,41,53–56] or born from a mother with a confirmed infection.[26,34] Postnatal cerebral evaluation relies on transfontanellar ultrasound primarily used as a fast screening tool, CT or MRI. Currently, the Brazilian government recommends a CT without contrast for all children with microcephaly; MRI is additionally performed according to clinical findings, as for example, in case of epilepsy or severe abnormal motor findings.[54] Major clinical and radiological findings are described in Table 2. Postnatal imaging generally correlates with prenatal observations. Diffuse cerebral calcifications, with preferential localization to the cortico-subcortical white matter junction, non-hypertensive ventriculomegaly, signs of abnormal migration and cortical development were the most frequent findings described. Dysgenesis of the cerebellum and brainstem was also observed. Additional findings include abnormal density of the white matter compatible with delayed myelinisation.[54,55] Finally, an abnormal skull with overridden bones and premature closure of the anterior fontanel were frequently observed. These findings are suggestive of the fetal brain disruption sequence that includes severe brain damage, leading to microcephaly with overlapping skull bones and prominence of the occipital bone plate, as well as excess scalp skin with a normal hair pattern,[57] all of which have been described in newborns with a suspected ZIKV infection. This sequence is thought to be due to brain insults occurring after 18 WG, as suggested by the normal hair pattern, and is associated with a poor prognosis, due to severe neurological impairment.[57] In a series of 20 cases presenting with this sequence, death occurred in seven because of aspiration pneumonia in the first year of life, and the remaining had severe developmental skills impairments.[57] Prognosis of the children reported here remains to be defined, as most of them are only currently a couple of months old.

RADIOLOGICAL MONITORING OF EXPOSED PREGNANCY

Basic ultrasound monitoring

Due to the high proportion of asymptomatic cases, ZIKV should be suspected in every pregnant woman with or without compatible symptoms living in or returning from countries with active ZIKV circulation (presented in Figure 1).[58] Moreover, it is currently unknown whether a prior maternal infection is protective, similarly to toxoplasmosis, or whether a re-infection can still be associated with fetal transmission, as observed in case of CMV infection. Laboratory diagnosis remains challenging and relies on molecular detection through RT-PCR or serological assays.[10,59,60] Baud *et al.*[59,60] recently proposed recommendations for adequate testing and monitoring in exposed pregnancies. A strong emphasis should be placed on basic ultrasound, as currently ZIKV is primarily circulating in countries where access to specialized materno-fetal centers is limited. Ultrasound monitoring is required independent of maternal testing because of the difficulties of diagnosis.[59] Similarly to other congenital infections, reports identified here suggest a significant delay between maternal infection and onset of fetal anomalies, enhancing the importance of regular ultrasound monitoring. In addition, it has been shown for other congenital infections that neurological lesions can rapidly evolve. As previously recommended, ultrasound should ideally be performed every 4 weeks starting from suspected exposure, and at least one ultrasound should be performed between 28–33 WG.[59] At this GA, the correlation between fetal HC and occipito-frontal circumference (OFC) at birth is the most accurate,[61] and cortical structures are developed enough to allow adequate evaluation of abnormal processes.[62,63] After 34 WG, skull ossification impairs the penetration of sound waves and adequate brain parenchymal evaluation. In cases of late exposure, even in the presence of normal ultrasound at this GA, monitoring should probably be maintained up to delivery because of the potential of late onset complications and the risk

Table 2 **Postnatal clinical and radiological findings of microcephalic newborns with a suspected ZIKV infection**

ZIKV newborn's status	Total cases screened	Cases with defect	(%)	Besnard *et al.*[27]	Schuler-Faccini *et al.*[2]	Culjat *et al.*[26]	Pernambuca Cohort[53–55]	Moron *et al.*[34]	Cavalheiro *et al.*[56]	Werner *et al.*[41]
Number of patients / study				*n* = 3	*n* = 35	*n* = 1	*n* = 104	*n* = 1	*n* = 42	*n* = 1
ZIKV fetus' status				Suspected	Confirmed 30?	Confirmed	Confirmed in 13	Confirmed	Suspected	Confirmed
Clinical observation										
General										
SGA or <2500 g	41	11	(27)	1	9	1	ud.	0	ud.	1
Microcephaly	158	158	(100)	3	35	1	104	1	13	?
<3 SD	144	100	(69)	3	25	1	70	1	ud.	
Arthrogryposis	41	4	(10)	0	4	0	ud.	0	ud.	0
Excess/redundant scalp skin	41	11	(27)	0	11	0	ud.	0	ud.	0
Overriding sutures	6	1	(20)	0	ud.	1	ud.	0	ud.	1
Sloping forehead	6	1	(20)	0	ud.	1	ud.	0	ud.	1
Clubfeet	41	5	(12)	0	5	0	ud.	0	ud.	0
Microophtalmy	41	1	(2)	0	1	0	ud.	0	ud.	0
Micropenis	41	1	(2)	1	0	1	ud.	0	ud.	0
Cryptorchidia	41	1	(2)	0	0	1	ud.	0	ud.	0
Hypospadias	41	1	(2)	0	0	1	ud.	0	ud.	0
Neurological examination							ud.			
Abnormal muscle tone	37	14	(38)	ud.	13	1	ud.	0	ud.	ud.
Hyperreflexia	37	7	(19)	ud.	7	0	ud.	0	ud.	ud.
Tremor	37	4	(11)	ud.	4	0	ud.	0	ud.	ud.
Irritability	38	7	(19)	ud.	7	0	ud.	0	ud.	1
Seizure	38	4	(11)	ud.	3	1	ud.	0	ud.	1
Primary reflex	5	0	(0)	ud.	ud.	Normal	ud.	Normal	ud.	ud.
Hearing	28	3	(11)	1	ud.	Normal	2/23	Normal	ud.	ud.
Ophthalmological examination										
Abnormal vision	5	2	(40)	1	ud.	1	ud.	0	ud.	ud.
Abnormal anterior segment	5	0	(0)	ud.	ud.	normal	ud.	0	ud.	ud.
Abnormal fundoscopic exam	70	20	(29)	ud.	11	1	8/33	0	ud.	ud.
Cerebral imaging										
Brain imaging method				MRI	CT	CT/MRI	CT/MRI	CT/MRI	CT	US/CT/MRI

(*Continues*)

Table 2 **(Continued)**

ZIKV newborn's status	Total cases screened	Cases with defect	(%)	Besnard *et al.*[27]	Schuler-Faccini *et al.*[2]	Culjat *et al.*[26]	Pernambuca Cohort[53–55]	Moron *et al.*[34]	Cavalheiro *et al.*[56]	Werner *et al.*[41]
General										
Overlapping bone sutures	102	14	(14)	0	0	0	0/58	1	13	1
Enlargement of the subarachnoid space	65	30	(47)	0	0	1	15/21	1	13	0
Calcifications (cortical, cerebellar, thalamus and basal ganglia)	102	88	(87)	0	20	1	54/58	1	12	1
Cortex										
Abnormal gyration	89	57	(65)	1	9	1	45/58	1	ud.	1
Diminution of cerebral mantle/cortical atrophy	102	34	(34)	0	0	1	20/23	1	13	1
Corpus callusum										
Dysgenesis/aplasia/hypoplasia	52	21	(41)	0	0	1	6/8	1	13	1
Ventricles										
Ventriculomegaly	102	70	(70)	0	12	1	43/58	1	13	1
Sub-ependymal pseudocyst	12	1	(4)	1	ud.	0	0/8	0	ud.	0
Intraventricular synechia	102	5	(5)	0	0	0	0	0	5	0
Hypertrophy choroid plexus	102	8	(8)	0	0	0	0	0	8	0
Cerebellum								0		
Hypoplasia/atrophia	90	22	(25)			1	21/46		0	0
Brainstem								0		
Hypotrophia/hypoplasia	90	10	(11)	0	0	0	9/46	0	0	0

This table presents major postnatal findings of nine studies. Among these cases, infection was confirmed in 16.[26,34,54,55] It is possible that infection was subsequently confirmed in 30 of the 35 cases reported by Schuler-Faccini *et al.*[2] through the isolation of specific IgM in the cerebrospinal fluid.[86] The reports from Hazin *et al.*,[55] de Fatima Vasco Aragao *et al.*[54] and the Microcephaly Epidemic Research Group[53] were analyzed together, as despite the fact that Hazin *et al.* and de Fatima Vasco Aragao *et al.* reported different cases,[87] they very likely all come from the same cohort of 104 children born in the state of Pernambuco in 2015, presented in the report by the Microcephaly Epidemic Research Group. Similarly, we cannot exclude that some of the cases reported by Cavalheiro *et al.*[56] were also part of this same cohort. Of note, in the report by Schuler-Faccini *et al.*, clinical information are available for 35 children, but imaging was so far only performed in 27. Similarly, so far, only 58 children of the Pernambuca cohort underwent imaging evaluation. In addition, clinical findings are available for three children from the report by Besnard *et al.*,[27] but only one of them had postnatal MRI.
Abnormal gyration refers to polymicrogyria and/or agyria and/or pachygyria.
ZIKV, Zika virus; SGA, small for gestational age; ud., undetermined.

of stillbirths.[33] Structures that should be evaluated in particular include biometrical parameters, placental thickness, size and shapes of ventricles, cerebellum, thalami and cavum septum pellucidum. In addition, sonographers should actively search for intraparenchymal, intraventricular or intraplacentar echodense foci or calcifications.[48] A transvaginal approach should be preferred at early GA or in case of cephalic presentation because of its higher performance.[64–66]

It should be emphasized that microcephaly remains a postnatal finding, which can only be suspected by prenatal evaluation. In addition, HC may not reflect an abnormal cerebral development and should therefore be evaluated with caution. Sloping of the forehead or associated additional brain lesions are suggestive of pathological microcephaly. In addition, most ultrasound devices provide calculators that refer to percentile and not standard deviation (SD). Microcephaly is defined as a postnatal OFC < -2nd SD adapted to GA and sex (i.e. below the 2.3 percentile) and severe microcephaly as an OFC < -3SD (i.e. below the 0.1 percentile).[67,68] As suggested by the International Society of Ultrasound in Obstetrics and Gynecology, microcephaly should only be suggested in case of an HC < -3SD[48]; in case of isolated microcephaly < -2SD, ultrasound should be repeated after 2–3 weeks prior to subsequent evaluation.[48] The presence of any anomalies, in particular calcifications, ventriculomegaly, pathological HC, namely, < -3SD, or with associated signs as described earlier, disruption of the cavum spetum pellucidum, abnormal morphology/size of the thalami and cerebellum should prompt subsequent specialized evaluations. Isolated IUGR should also alert sonographers.

Fetal neurosonogram or MRI

These highly specialized techniques enable a better description of fetal brain structures. They have been shown to be extremely useful in the evaluation of gyration disorders, as frequently observed in ZIKV-infected fetuses.[62,63] Although MRI was initially thought to be superior, the significant progress in ultrasound techniques, in particular 3D examinations, has increased its sensitivity and therefore may now be considered as a sufficient examination.[62,63] In addition to the cost advantage, neurosonogram offers a better characterization of calcifications and is better tolerated than MRI. Nevertheless, MRI is not influenced by the mother's body mass index, amniotic fluid volume or fetal presentation. Indeed, in case of cephalic presentation, a transvaginal approach may be required to perform optimal neurosonogram.[69] As mentioned earlier, the sensitivity of the ultrasound decreases after 34 WG because of skull ossification. The ideal period to evaluate gyration disorder is between 28–32 WG for both techniques.[62,63]

Fetal MRI has been shown to increase the positive predictive value of diagnosis of fetal brain anomalies in comparison with ultrasound alone, for example, in case of congenital CMV infection.[70] However, fetal MRI has a lower negative predictive value than ultrasound and may be associated with false positive results.[70] Therefore, caution should be taken in the presence of brain anomalies identified by fetal MRI alone. Considering earlier, fetal neurosonogram is probably the ideal investigation prior to 34 WG. It can be complemented by fetal MRI in cases of ambiguous results or if TOP is considered, to help parental decision.

DISCUSSION AND PERSPECTIVES

Current reports highlight the specific cerebral tropism of ZIKV. The pattern of cerebral anomalies is highly destructive and mimics the most severe anomalies found in congenital CMV or LCMV and to a lesser extent toxoplasmosis infections. No other systemic malformations seem to be induced, unlike CMV or toxoplasmosis infections. The specificity of neurological anomalies is also observed in LCMV infections, in which the only additional anomalies described were hydrops fetalis and IUGR, as currently described for ZIKV. Nevertheless, the diagnosis of TORCH infections cannot rely on radiological evaluation alone.[71]

Zika virus prenatal infection is associated with severe cerebral lesions associated with a dismal prognosis. Most of them are related to severe early injuries to the developing brain, which depends on three distinct essential processes.[72] First is the neuronal proliferation occurring between the 2nd and 4th months of gestation that gives rise to both neurons and glial cells, followed by neuronal migration during 3rd–5th month of gestation and finally cortical organization through cell differentiation and laminar/columnar organization.[72] This final step starts around the 22nd WG and will continue in the postnatal life.[72] Current experimental *in vitro* studies[23,73] demonstrated the ability of ZIKV to alter all of these processes, first by inducing the mortality of the neural progenitor cells[23,73] and subsequently by altering cortical development and maturation.[23] Despite the severity of ZIKV-associated cerebral lesions, both suggested by these experimental studies and the fetal cases reported here, caution should be taken on these preliminary conclusions, as current reports may only reflect the most severe forms of the disease.

Extreme caution should be taken when counseling parents of exposed fetuses. So far, few cases have been well described, and the recommendations described here may become obsolete. The sole presence of fetal microcephaly should not be considered as a marker of ZIKV infection as, both in Brazil and French Polynesia, all newborns with a confirmed infection had additional cerebral lesions on postnatal imaging.[2] It should probably not be considered as justification for a TOP alone, due to the lack of reliability of prenatal measurements, as discussed previously.[61] Similarly, isolated ventriculomegaly, brain calcifications or sub-ependymal pseudoycsts have been associated with a favorable prognosis.[51,74,75] A similar prognosis might be suspected for isolated anomalies, even in case of ZIKV exposition, but further studies are needed. Currently, prognosis of infected fetuses remains difficult to establish because of lack of follow-up studies and knowledge of disease's spectrum. Further studies are urgently needed.[13] With increasing knowledge, it is likely that additional prognostic factors may develop such as maternal or amniotic

fluid viral load, which could assist in parental decision-making. Finally, it is extremely important to remember that ZIKV is currently affecting countries in which abortion remains illegal or poorly accepted.[76] In that countries, the benefit of screening is highly questionable, especially as it may lead to illegal and inadequate TOP.[77] Nevertheless, a late 3rd trimester should be at least proposed to decide on location of delivery.

ACKNOWLEDGEMENTS

We warmly thank Kirsten Niles and Karine Lepigeon for computer assistance and/or critical review of the manuscript.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Zika virus has emerged as novel teratogenic infectious agent.
- It is urgent to better define the spectrum of anomalies observed in infected fetuses.

WHAT DOES THIS STUDY ADD?

- We provide a comprehensive analysis of all cases to date to highlight specific prenatal and postnatal radiological findings of congenital Zika virus infection.
- We provide recommendations for adequate radiological monitoring of at-risk pregnancies.

REFERENCES

1. Cooper LZ. The history and medical consequences of rubella. Rev Infect Dis 1985;7(Suppl 1):S2–10.
2. Schuler-Faccini L, Ribeiro EM, Feitosa IML, *et al.* Possible association between Zika virus infection and microcephaly – Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016;65:59–62.
3. Gardner LM, Chen N, Sarkar S. Global risk of Zika virus depends critically on vector status of *Aedes albopictus*. Lancet Infect Dis 2016;16:522–3.
4. Musso D, Stramer SL. AABB Transfusion-transmitted Diseases Committee, Busch MP, International Society of Blood Transfusion Working Party on Transfusion-transmitted Infectious Diseases. Zika virus: a new challenge for blood transfusion. Lancet Lond Engl 2016;387:1993–4.
5. D'Ortenzio E, Matheron S, de Lamballerie X, *et al.* Evidence of sexual transmission of Zika virus. N Engl J Med 2016;374(22):2195–8, DOI: 10.1056/NEJMc1604449.
6. Dupont-Rouzeyrol M, Biron A, O'Connor O, *et al.* Infectious Zika viral particles in breastmilk. Lancet Lond Engl 2016;387:1051.
7. Musso D, Roche C, Nhan T-X, *et al.* Detection of Zika virus in saliva. J Clin Virol 2015;68:53–5.
8. Liuzzi G, Nicastri E, Puro V, *et al.* Zika virus in saliva – new challenges for prevention of human to human transmission. Eur J Intern Med 2016, DOI: 10.1016/j.ejim.2016.04.022.
9. Dick GWA, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg 1952;46:509–20.
10. Musso D, Gubler DJ. Zika virus. Clin Microbiol Rev 2016;29:487–524.
11. Panchaud A, Stojanov M, Ammerdorffer A, *et al.* Emerging role of Zika virus in adverse fetal and neonatal outcomes. Clin Microbiol Rev 2016;29:659–94.
12. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects – reviewing the evidence for causality. N Engl J Med 2016;374:1981–7.
13. Panchaud A, Vouga M, Musso D, Baud D. An International Registry for Women Exposed to Zika Virus during Pregnancy: time for answers. Lancet Infect Dis 2016 (*In press*).
14. Messina JP, Kraemer MU, Brady OJ, *et al.* Mapping global environmental suitability for Zika virus. eLife 2016;5.
15. Malinger G, Werner H, Rodriguez Leonel JC, *et al.* Prenatal brain imaging in congenital toxoplasmosis. Prenat Diagn 2011;31:881–6.
16. Yinon Y, Farine D, Yudin MH. Screening, diagnosis, and management of cytomegalovirus infection in pregnancy. Obstet Gynecol Surv 2010;65:736–43.
17. Guerra B, Simonazzi G, Banfi A, *et al.* Impact of diagnostic and confirmatory tests and prenatal counseling on the rate of pregnancy termination among women with positive cytomegalovirus immunoglobulin M antibody titers. Am J Obstet Gynecol 2007;196:221.e1–221.e6.
18. Berrebi A, Kobuch WE, Bessieres MH, *et al.* Termination of pregnancy for maternal toxoplasmosis. Lancet Lond Engl 1994;344:36–9.
19. Besnard M, Lastere S, Teissier A, *et al.* Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill Bull 2014;19(13), PII: 20751.
20. Oliveira Melo AS, Malinger G, Ximenes R, *et al.* Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol 2016;47:6–7.
21. Calvet G, Aguiar RS, Melo ASO, *et al.* Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis 2016;16(6):653–60, DOI: 10.1016/S1473-3099(16)00095-5.
22. Wu K-Y, Zuo G-L, Li X-F, *et al.* Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. Cell Res 2016;26:645–54.
23. Cugola FR, Fernandes IR, Russo FB, *et al.* The Brazilian Zika virus strain causes birth defects in experimental models. Nature 2016;534:267–71.
24. de Miranda-Filho DB, Martelli CMT, de Ximenes RAA, *et al.* Initial description of the presumed congenital Zika syndrome. Am J Public Health 2016;106:598–600.
25. McCarthy M. Severe eye damage in infants with microcephaly is presumed to be due to Zika virus. BMJ 2016;352:i855.
26. Culjat M, Darling SE, Nerurkar VR, *et al.* Clinical and Imaging findings in an infant with Zika embryopathy. Clin Infect Dis 2016, PII: ciw324.
27. Besnard M, Eyrolle-Guignot D, Guillemette-Artur P, *et al.* Congenital cerebral malformations and dysfunction in fetuses and newborns following the 2013 to 2014 Zika virus epidemic in French Polynesia. Euro Surveill Bull 2016;21(13), DOI: 10.2807/1560-7917.ES.2016.21.13.30181.
28. Cauchemez S, Besnard M, Bompard P, *et al.* Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study. Lancet 2016;387(10033):2125–32, DOI: 10.1016/S0140-6736(16)00651-6.
29. Johansson MA, Mier-Y-Teran-Romero L, Reefhuis J, *et al.* Zika and the risk of microcephaly. N Engl J Med 2016;375(1):1–4, DOI: 10.1056/NEJMp1605367.
30. Kleber de Oliveira W, Cortez-Escalante J, De Oliveira WTGH, *et al.* Increase in reported prevalence of microcephaly in infants born to women living in areas with confirmed Zika virus transmission during the first trimester of pregnancy – Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016;65:242–7.
31. Jaenisch T, Rosenberger K, Brady O, *et al.* Estimating the risk for microcephaly after Zika virus infection in Brazil. Bull World Health Organ 2016published online May 30. DOI: 10.1056/NEJMoa1604037.
32. Pacheco O, Beltrán M, Nelson CA, *et al.* Zika virus disease in Colombia – preliminary report. N Engl J Med 2016, DOI: 10.1056/NEJMoa1604037.
33. Brasil P, Pereira JP, Raja Gabaglia C, *et al.* Zika virus infection in pregnant women in Rio de Janeiro – preliminary report. N Engl J Med 2016.
34. Moron A, Cavalheiro S, Milani H, *et al.* Microcephaly associated with maternal Zika virus infection. BJOG 2016;123(8):1265–9, DOI: 10.1111/1471-0528.14072.
35. Mlakar J, Korva M, Tul N, *et al.* Zika virus associated with microcephaly. N Engl J Med 2016;374:951–8.

36. Jouannic J-M, Friszer S, Leparc-Goffart I, *et al.* Zika virus infection in French Polynesia. The Lancet 2016;387:1051–2.
37. Guillemette-Artur P, Besnard M, Eyrolle-Guignot D, *et al.* Prenatal brain MRI of fetuses with Zika virus infection. Pediatr Radiol 2016;46 (7):1032–9, DOI: 10.1007/s00247-016-3619-6.
38. Sarno M, Sacramento GA, Khouri R, *et al.* Zika virus infection and stillbirths: a case of hydrops fetalis, hydranencephaly and fetal demise. PLoS Negl Trop Dis 2016;10:e0004517.
39. Werner H, Fazecas T, Guedes B, *et al.* Intrauterine Zika virus infection and microcephaly: correlation of perinatal imaging and three-dimensional virtual physical models. Ultrasound Obstet Gynecol 2016;47:657–60.
40. Driggers RW, Ho C-Y, Korhonen EM, *et al.* Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. N Engl J Med 2016;374(22):2142–51. DOI: 10.1056/NEJMoa1601824.
41. Werner H, Sodré D, Hygino C, *et al.* First trimester intrauterine Zika virus infection and brain pathology: prenatal and postnatal neuroimaging findings. Prenat Diagn 2016;36(8):785–9, DOI: 10.1002/pd.4860.
42. Perez S, Tato R, Cabrera JJ, *et al.* Confirmed case of Zika virus congenital infection, Spain, March 2016. Euro Surveill 2016;21(24), DOI: 10.2807/1560-7917.ES.2016.21.24.30261.
43. Malinger G, Lev D, Lerman-Sagie T. Imaging of fetal cytomegalovirus infection. Fetal Diagn Ther 2011;29:117–26.
44. Hohlfeld P, MacAleese J, Capella-Pavlovski M, *et al.* Fetal toxoplasmosis: ultrasonographic signs. Ultrasound Obstet Gynecol 1991;1:241–4.
45. Bonthius DJ, Wright R, Tseng B, *et al.* Congenital lymphocytic choriomeningitis virus infection: spectrum of disease. Ann Neurol 2007;62:347–55.
46. Bonthius DJ. Lymphocytic choriomeningitis virus: an under-recognized cause of neurologic disease in the fetus, child, and adult. Semin Pediatr Neurol 2012;19:89–95.
47. Cuiller F, Avignon M. Case of the week # 168. The Fetus.net. https://sonoworld.com/TheFetus/Case.aspx?CaseId=1696&answer=1.
48. Papageorghiou AT, Thilaganathan B, Bilardo CM, *et al.* ISUOG interim guidance on ultrasound for Zika virus infection in pregnancy: information for healthcare professionals. Ultrasound Obstet Gynecol 2016;47:530–2.
49. França GVA, Schuler-Faccini L, Oliveira WK, *et al.* Congenital Zika virus syndrome in Brazil: a case series of the first 1501 live births with complete investigation. Lancet 2016, DOI: 10.1016/S0140-6736 (16)30902-3.
50. Malinger G, Lev D, Ben Sira L, *et al.* Congenital periventricular pseudocysts: prenatal sonographic appearance and clinical implications. Ultrasound Obstet Gynecol 2002;20:447–51.
51. Esteban H, Blondiaux E, Audureau E, *et al.* Prenatal features of isolated subependymal pseudocysts associated with adverse pregnancy outcome. Ultrasound Obstet Gynecol 2015;46:678–87.
52. Adibi JJ, Marques ETA, Cartus A, Beigi RH. Teratogenic effects of the Zika virus and the role of the placenta. Lancet Lond Engl 2016;387:1587–90.
53. Microcephaly Epidemic Research Group1. Microcephaly in infants, Pernambuco State, Brazil, 2015. Emerg Infect Dis 2016;22(6):1090–3. DOI: 10.3201/eid2206.160062.
54. de Fatima Vasco Aragao M, van der Linden V, Brainer-Lima AM, *et al.* Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study. BMJ 2016;353:i1901.
55. Hazin AN, Poretti A, Cruz DDCS, *et al.* Computed tomographic findings in microcephaly associated with Zika virus. N Engl J Med 2016;374 (22):2193–5, DOI: 10.1056/NEJMc1603617.
56. Cavalheiro S, Lopez A, Serra S, *et al.* Microcephaly and Zika virus: neonatal neuroradiological aspects. Childs Nerv Syst 2016;32 (6):1057–60, DOI: 10.1007/s00381-016-3074-6.
57. Corona-Rivera JR, Corona-Rivera E, Romero-Velarde E, *et al.* Report and review of the fetal brain disruption sequence. Eur J Pediatr 2001;160:664–7.
58. Centers for Disease Control and Prevention. Zika travel information. 2016; published online May 26. http://wwwnc.cdc.gov/travel/page/zika-travel-information (accessed June 11, 2016).
59. Baud D, Van Mieghem T, Musso D, *et al.* Clinical management of pregnant women exposed to Zika virus. Lancet Infect Dis 2016, DOI: 10.1016/S1473-3099(16)30008-1.
60. Vouga M, Musso D, Panchaud A, Baud D. Clinical mangament of pregnant women exposed to Zika virus: an update. Lancet Infect Dis 2016;16(7):773.
61. Leibovitz Z, Daniel-Spiegel E, Malinger G, *et al.* Microcephaly at birth – the accuracy of three references for fetal head circumference. How can we improve prediction? Ultrasound Obstet Gynecol 2016;47(5):586–92. DOI: 10.1002/uog.15801.
62. Denis D, Maugey-Laulom B, Carles D, *et al.* Prenatal diagnosis of schizencephaly by fetal magnetic resonance imaging. Fetal Diagn Ther 2001;16:354–9.
63. Rubod C, Robert Y, Tillouche N, *et al.* Role of fetal ultrasound and magnetic resonance imaging in the prenatal diagnosis of migration disorders. Prenat Diagn 2005;25:1181–7.
64. Lim J, Whittle WL, Lee Y-M, *et al.* Early anatomy ultrasound in women at increased risk of fetal anomalies. Prenat Diagn 2013;33:863–8.
65. Van Mieghem T, Hindryckx A, Van Calsteren K. Early fetal anatomy screening: who, what, when and why? Curr Opin Obstet Gynecol 2015;27:143–50.
66. Engels AC, Joyeux L, Brantner C, *et al.* Sonographic detection of central nervous system defects in the first trimester of pregnancy. Prenat Diagn 2016;36:266–73.
67. Ashwal S, Michelson D, Plawner L, Dobyns WB. Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Practice parameter: evaluation of the child with microcephaly (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2009;73:887–97.
68. Villar J, Cheikh Ismail L, Victora CG, *et al.* International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-sectional Study of the INTERGROWTH-21st Project. Lancet Lond Engl 2014;384:857–68.
69. International Society of Ultrasound in Obstetrics & Gynecology Education Committee. Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination' and the 'fetal neurosonogram'. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2007;29:109–16.
70. Benoist G, Salomon LJ, Mohlo M, *et al.* Cytomegalovirus-related fetal brain lesions: comparison between targeted ultrasound examination and magnetic resonance imaging. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2008;32:900–5.
71. Beke A, Latkóczy K, Nagy GR, *et al.* Comparison of prevalence of toxoplasma and cytomegalovirus infection in cases with fetal ultrasound markers in the second trimester of pregnancy. Prenat Diagn 2011;31:945–8.
72. Razek AAKA, Kandell AY, Elsorogy LG, *et al.* Disorders of cortical formation: MR imaging features. Am J Neuroradiol 2009;30:4–11.
73. Tang H, Hammack C, Ogden SC, *et al.* Zika virus infects human cortical neural progenitors and attenuates their growth. Cell Stem Cell 2016;18:587–90.
74. Scala C, Familiari A, Pinas A, *et al.* Perinatal and long-term outcome in fetuses diagnosed with isolated unilateral ventriculomegaly: systematic review and meta-analysis. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2016, DOI: 10.1002/uog.15943.
75. Dhombres F, Friszer S, Maurice P, *et al.* Prognosis of fetal parenchymal cerebral lesions without ventriculomegaly in congenital toxoplasmosis infection. Fetal Diagn Ther 2016, DOI: 10.1159/000445113.
76. Roa M. Zika virus outbreak: reproductive health and rights in Latin America. Lancet Lond Engl 2016;387:843.
77. Aiken ARA, Scott JG, Gomperts R, *et al.* Requests for abortion in Latin America related to concern about Zika virus exposure. N Engl J Med 2016;375(4):396–8, DOI: 10.1056/NEJMc1605389.
78. Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Trans R Soc Trop Med Hyg 1954;48:139–45.
79. Duffy MR, Chen T-H, Hancock WT, *et al.* Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–43.
80. Tognarelli J, Ulloa S, Villagra E, *et al.* A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol 2016;161(3):665–8. DOI: 10.1007/s00705-015-2695-5.

81. Cardoso CW, Paploski IAD, Kikuti M, *et al.* Outbreak of exanthematous illness associated with Zika, Chikungunya, and dengue viruses, Salvador, Brazil. Emerg Infect Dis 2015;21:2274–6.
82. Ministério da Saúde divulga boletim epidemiológico. Portal Saúde – Minist. Saúde – Wwwsaudegovbr. http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/20805-ministerio-da-saude-divulga-boletim-epidemiologico (accessed June 2, 2016).
83. European Centre for Disease Prevention and Control. Rapid risk assesment : microcephaly in Brazil potentially linked to the Zika virus epidemic. 2015; published online Nov 24. http://ecdc.europa.eu/en/publications/Publications/zika-microcephaly-Brazil-rapid-risk-assessment-Nov-2015.pdf (accessed June 18, 2016).
84. Pan American Health Organization. Epidemiological alert – neurological syndrome, congenital malformations and Zika virus infection. Implications for public health in the Americas. 2015; published online Dec 1.
85. World Health Organization. WHO statement on the first meeting of the Internation Health Regulations (2005) (IHR2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations. 2016; published online Feb 1. http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/ (accessed June 18, 2016).
86. Cordeiro MT, Pena LJ, Brito CA, *et al.* Positive IgM for Zika virus in the cerebrospinal fluid of 30 neonates with microcephaly in Brazil. Lancet 2016;387(10030):1811–2, DOI: 10.1016/S0140-6736(16)30253-7.
87. Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study. BMJ 2016;353:i3182.

Appendix 1