Pathology of congenital Zika syndrome in Brazil: a case series


Summary

Background Zika virus is an arthropod-borne virus that is a member of the family Flaviviridae transmitted mainly by mosquitoes of the genus *Aedes*. Although usually asymptomatic, infection can result in a mild and self-limiting illness characterised by fever, rash, arthralgia, and conjunctivitis. An increase in the number of children born with microcephaly was noted in 2015 in regions of Brazil with high transmission of Zika virus. More recently, evidence has been accumulating supporting a link between Zika virus and microcephaly. Here, we describe findings from three fatal cases and two spontaneous abortions associated with Zika virus infection.

Methods In this case series, formalin-fixed paraffin-embedded tissue samples from five cases, including two newborn babies with microcephaly and severe arthrogryposis who died shortly after birth, one 2-month-old baby, and two placentas from spontaneous abortions, from Brazil were submitted to the Infectious Diseases Pathology Branch at the US Centers for Disease Control and Prevention (Atlanta, GA, USA) between December, 2015, and March, 2016. Specimens were assessed by histopathological examination, immunohistochemical assays using a mouse anti-Zika virus antibody, and RT-PCR assays targeting the NS5 and envelope genes. Amplicons of RT-PCR positive cases were sequenced for characterisation of strains.

Findings Viral antigens were localised to glial cells and neurons and associated with microcalfications in all three fatal cases with microcephaly. Antigens were also seen in chorionic villi of one of the first trimester placentas. Tissues from all five cases were positive for Zika virus RNA by RT-PCR, and sequence analyses showed highest identities with Zika virus strains isolated from Brazil during 2015.

Interpretation These findings provide strong evidence of a link between Zika virus infection and different congenital central nervous system malformations, including microcephaly as well as arthrogryposis and spontaneous abortions.

Funding None.

Introduction Zika virus is an arthropod-borne virus transmitted primarily by mosquitoes of the genus *Aedes*. The virus belongs to the Flaviviridae family, which includes other flaviviruses such as dengue, yellow fever, West Nile, and Japanese encephalitis viruses. Zika virus was first isolated in the Zika Forest of Uganda in 1947. Before 2007, sporadic small clusters of human infections in Africa and Asia were reported. The geographical range of several arboviruses is expanding with increased travel and climate change. Since 2007, several large Zika virus outbreaks have occurred, such as those in Micronesia and French Polynesia, and in March, 2015, autochthonous virus transmission was first detected in Brazil. The Zika virus has rapidly spread through South and Central America, and the Caribbean. Although the virus is expected to continue to spread, there are uncertainties around where and how the virus will spread over time.

Illness resulting from Zika virus infection is typically mild and self-limiting. However, since 2013, an increased incidence of neurological symptoms, including Guillain-Barré syndrome, have been described following Zika virus infection. Furthermore, the Zika virus outbreak in Brazil in 2015 has caused worldwide concern because of the association with increased rates of microcephaly in newborn babies as well as severe ocular abnormalities. Autochthonous circulation of Zika virus has been described in 25 Brazilian states with 7343 suspect cases of microcephaly being investigated. Of these 1271, including 57 fatal cases, were confirmed to have microcephaly or other CNS abnormalities; mothers of some of these infants had clinical signs consistent with Zika virus infection during pregnancy, but most were not tested for Zika virus infection. Before the Brazilian outbreak, only two cases of perinatal transmission were described in French Polynesia. However, a retrospective analysis of cases from French Polynesia—subsequent to the 2015 Brazil outbreak—reported an increase of CNS malformations in fetuses and infants following the Zika virus outbreak in 2013–14. Zika virus genome was detected in the amniotic fluid samples from two pregnant women from Brazil whose fetuses had been diagnosed with microcephaly. Autopsies of two fetuses after elective termination of pregnancy showed microcephaly, and the cause was identified as Zika virus by RT-PCR assay and electronic microscopy. Zika virus has been recently found to target human
brain cells in pluripotent stem cells cultured as neural cells, neurospheres, and brain organoids. No detailed report on Zika virus immunolocalisation in tissues has been described.

In addition to genetic, toxic, iatrogenic, and other non-infectious causes, congenital malformations can be caused by the vertical transmission of viruses, bacteria, and parasites such as cytomegalovirus, herpes simplex virus, rubella virus, Toxoplasma gondii, and Treponema pallidum. Infection during the first trimester of pregnancy is of particular concern with most of these agents and can be associated with serious consequences. On the other hand, cytomegalovirus infection at any point during pregnancy can result in severe CNS sequelae. Severe congenital abnormalities have been presumably related to Zika virus infection; however, only a few cases have been described and definitive diagnosis of Zika virus infection is needed to support this association.

Here, we aimed to describe findings from three fatal cases (two newborn babies and a 2-month-old baby) and two spontaneous abortions associated with Zika virus infection. Preliminary findings of the five cases reported here have previously been summarised in the Morbidity and Mortality Weekly Report.

Methods
Case histories
In this case series, formalin-fixed paraffin-embedded tissues sample from five cases, including two newborn babies with microcephaly and severe arthrogryposis who died shortly after birth, one 2-month-old baby, and two placentas from spontaneous abortions, from Brazil were submitted to the Infectious Diseases Pathology Branch at the US Centers for Disease Control and Prevention (Atlanta, GA, USA) between December, 2015, and March, 2016. Case 1 was a boy born at 36 weeks’ gestation, who died 20 h after birth. Ultrasonography done in the second trimester showed microcephaly, as well as limb and genital malformations. His 26-year-old mother had developed fever and rash during the first trimester of pregnancy and no laboratory studies were done. No drug or chemical exposures were reported. At birth, microcephaly and upper and lower limb malformations were noted. Formalin-fixed paraffin-embedded (FFPE) tissues from the newborn baby were available for assessment from the brain, heart, liver, spleen, kidney, and cartilage.

Case 2 was a girl born at 38 weeks’ gestation, who died 6 h after birth. Her 19-year-old mother had developed fever and rash during the first 4 weeks of pregnancy and
no laboratory studies were done. No drug or chemical exposures were reported. No malformations were detected by ultrasonography done at 20 weeks' gestation. At birth, microcephaly and upper and lower limb malformations were noted. FFPE tissues were available for examination from the placenta, umbilical cord, chorionic membranes, brain, heart, liver, lung, spleen, and kidney.

Case 3 was a girl born at 38 weeks' gestation. Her 16-year-old mother developed symptoms of fever, rash, and body aches during the first trimester of pregnancy. No drug or chemical exposures were reported. Microcephaly and upper and lower limb malformations were detected on ultrasonography in the fifth month of pregnancy. The baby was referred to the neonatal intensive care unit immediately after birth. Apgar scores were 7 and 8 (weight of 2208 g and head circumference of 29 cm). She had seizures within the first 24 h of life and required mechanical ventilation. CT scans at 2 weeks after birth showed lissencephaly and hydrocephalus. Serological tests for cytomegalovirus (IgM and IgG), rubella virus (IgM), and Toxoplasma gondii (IgM and IgG) were negative. Because of status epilepticus and seizures that were difficult to control, the baby was maintained on mechanical ventilation; attempts at extubation failed. She acquired an infection refractory to antibiotic therapy. Her condition worsened and she developed a coagulopathy requiring multiple transfusions but did not recover and suffered multiorgan failure. She died 2 months after birth. FFPE tissues were available from the brain, kidney, spleen, adrenal gland, lung, liver, and heart.

Case 4 was a previously healthy 37-year-old woman who presented with fever and rash in week 8 of pregnancy and subsequently had a spontaneous abortion at 11 weeks' gestation. No drug or chemical exposures were reported. Antenatal serological testing was negative for cytomegalovirus, rubella virus, Toxoplasma gondii, herpes simplex virus, and HIV. FFPE blocks of placenta were available for assessment.

Case 5 was a previously healthy 31-year-old woman who presented with fever and rash at 8 weeks of pregnancy, but no laboratory studies were done. No drug or chemical exposures were reported. She had a spontaneous abortion at 13 weeks' gestation. Ultrasonography at 6 weeks' gestation showed no abnormalities, and antenatal serological tests for HIV, herpes simplex virus, hepatitis B and C viruses, cytomegalovirus (IgM), rubella virus (IgM), and Toxoplasma gondii were negative, and bloodwork showed a haematocrit of 41%, platelet count of 231000 cells per mm$^3$, and glucose concentration of 84 mg/dL. Ultrasonography at 13 weeks' gestation showed a fetus small for gestational age (length 17 mm, yolk sac diameter 46 mm), and with no detectable heartbeat. FFPE blocks from the curettage specimen were available for examination.

**Sample collection**

All pregnant women had fever and rash in the first trimester of pregnancy. Four patients resided in the region of Rio Grande do Norte, Brazil, and one patient resided in the region of Piauí, Brazil. FFPE tissues from all cases were submitted to the Infectious Diseases Pathology Branch at the US Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) For diagnostic consultation. Medical records and preliminary autopsy reports were collected when available. All samples and associated medical and autopsy records were provided in the context of diagnostic consultation, a routine public health service provided by the CDC. As such, institutional review was not required for the testing described in this report. Informed consent and permission to present individual case reports were obtained from the patients (cases 4 and 5) or the patients’ family (cases 1, 2, and 3).

**RT-PCR assays**

RNA was extracted from FFPE tissues using a phenol-chloroform extraction protocol, as previously described,$^{24}$ and assessed by two Zika virus specific RT-PCR assays targeting the NS5 and envelope genes.$^{25}$ All positive amplicons were directly sequenced for confirmation and further characterisation of strains. All samples were also tested by dengue specific RT-PCR assay as previously described.$^{26}$

**Histopathology and immunohistochemistry**

Routine haematoxylin-eosin stains were done for histopathological assessment. An immunohistochemical assay for Zika virus was done using a mouse polyclonal anti-Zika virus antibody and a polymer-based indirect colorimetric immunoalkaline phosphatase detection system with fast red chromogen (Thermo Fisher Scientific, Runcom, Cheshire, UK). Deparaffinised and rehydrated tissue sections were placed in a LAB Vision autostainer and digested in 0.1 mg/mL proteinase K (Roche Diagnostics GmbH, Mannheim, Germany). Tissue sections were incubated with anti-Zika antibody for 30 min followed by sequential incubations with MACH 4Universal AP Polymer Kit (Biocare Medical LLC, Concord, CA, USA) and fast red substrate (Dako North America, Carpinteria, CA, USA). Sections were then counterstained in Mayer’s modified haematoxylin (Poly Scientific R&D Corp., Bay Shore, NY, USA) using the Sakura Automatic Slide Stainer and mounted with aqueous mounting medium (Lerner Laboratories, Pittsburg, PA, USA). The optimal dilution of anti-Zika virus antibody for the immunohistochemical assay was determined (1/500) by using a series of titrations applied to sections from FFPE. Vero E6 kidney cells that had been inoculated with Zika virus and harvested, fixed in formalin, and embedded in paraffin were used as a positive control. Antibody specificity was assessed by testing on normal placentas (n=10), placentas with non-Zika virus infections,$^7$ and brain tissues obtained at
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autopsy from five patients who died of a non-infectious cause and from six patients who died of confirmed viral or bacterial infection other than Zika virus infection. The Zika virus antibody cross-reacted with dengue virus, but not with other flaviviridae, including yellow fever, West Nile, and Japanese encephalitis viruses. Negative controls were also run in parallel and consisted of sequential tissue sections from case patients each incubated with normal mouse serum. Common congenital infections, including rubella virus, herpes simplex viruses types 1 and 2, *Toxoplasma gondii*, cytomegalovirus, and *Treponema* species, were ruled out in all cases by immunohistochemical assays.

**Role of the funding source**

There was no funding source for this study. All authors had full access to the data and SRZ and RBM had responsibility for submission of the report.

**Results**

At autopsy, case 1 had a bodyweight of 2740 g and length of 41 cm, a head circumference of 29 cm, a thoracic circumference of 34 cm, and an abdominal circumference of 31 cm. External examination of the body showed subcutaneous oedema, microcephaly, craniofacial malformations (hypertelorism, flat midface, low nasal bridge, and short nose), severe arthrogryposis with upper and lower limb malformations with joint contractures, and craniosynostosis (figure 1A, B). Cryptorchidism was also noted. Examination of CNS showed lissencephaly, alobar holoprosencephaly, and cerebellar hypoplasia. The chest examination showed bilateral pulmonary hypoplasia that was confirmed by radial alveolar counts. Histopathological changes were restricted to the brain, and included parenchymal calcification, microglial nodules, gliosis, neuronal and glial cell degeneration, and necrosis. Histopathological findings in the other organs were non-specific. Immunohistochemical testing of the brain was positive for Zika virus in neural cells and areas of microcalcification. No immunohistochemical evidence of Zika virus in the heart, liver, spleen, kidney, and cartilage was noted. Zika virus RT-PCR assays were positive only in brain tissues, and sequence analysis showed 99–100% identity with Zika virus strains isolated from Brazil during 2015. RT-PCR assays were negative for dengue viruses.

At autopsy, case 2 had a bodyweight of 2550 g and length of 34 cm, a head circumference of 31 cm, a thoracic circumference of 32 cm, and an abdominal circumference of 31 cm. She had microcephaly and craniofacial malformations, including hypertelorism, flat midface, low nasal bridge and short nose, severe contracture of joints, levoscoliosis (figure 1C), and craniosynostosis. Gross examination of the brain showed normal division of hemispheres, lissencephaly, and ventriculomegaly with lateral ventricle hydrocephalus and cerebellar hypoplasia (figure 1D). Gross examination of the thoracic cavity showed pulmonary hypoplasia that was confirmed by radial alveolar counts. The umbilical cord showed only one umbilical artery. No other major features of the placenta and chorionic membranes were seen. Histopathological examination of the brain showed substantial gliosis, neuronal and glial cell degeneration, necrosis, and microcalcifications. The lung tissue showed hypoplasia and intra-alveolar haemorrhage. Other organs showed no major

Figure 1: Congenital Zika syndrome

(A, B) Case 1 showing craniofacial malformations, including microcephaly, flat nasal bridge, anteverted nares, and features of fetal akinesia deformation sequence with upper and lower limb contractures and valgus deformities.

(C) Case 2 at autopsy showing levoscoliosis and gross examination of the brain (D) displaying two underdeveloped hemispheres with lissencephaly (short arrow) and ventriculomegaly (arrowheads) of the lateral ventricle. Cerebellar hypoplasia is also evident in the exposed left cerebellar hemisphere (long arrow).
abnormalities. Placenta showed fibrosis, calcification, and deposits of fibrinoid material consistent with third trimester gestation. No evidence of inflammation was noted. Immunolocalisation of viral antigens was seen in degenerating glial cells and neurons and in the vicinity of microcalcifications. Zika virus antigens were not observed in the heart, liver, lung, kidney, placenta, chorionic membranes, or umbilical cord. Zika virus RT-PCR assays were positive only in brain tissues and sequence analysis showed 99–100% identity with Zika virus strains isolated from Brazil during 2015. RT-PCR assays were negative for dengue viruses. At microscopic examination of autopsy tissues, the brain from case 3 showed cerebral atrophy with diffuse gliosis and prominent calcification arranged in a subcortical band-like pattern and also as scattered foci throughout the parenchyma (figure 2A–C). Immunolocalisation of viral antigens was seen in degenerating glial cells and neurons and in the vicinity of microcalcifications (figure 2D–F). No immunostaining was observed in visceral organs. Zika virus RT-PCR assays were positive in brain tissues and sequence analysis showed 99–100% identity with Zika virus strains isolated from Brazil during 2015. RT-PCR assays were negative for dengue viruses. The leptomeninges showed vascular congestion and a mixed acute and chronic inflammatory infiltrate. Abundant Gram-negative rod bacteria were seen in the meninges associated with acute inflammation. Gram-negative rod bacteria were also scattered throughout the brain. Evidence of Gram-negative bacterial sepsis in each of the visceral organs was noted, and the lungs showed diffuse congestion and interstitial expansion by a mixed acute and chronic inflammatory infiltrate. Other organs showed minimal non-specific changes. Pan-eubacterial 16S rRNA PCR testing detected \textit{Serratia marcescens} in the brain and visceral organ blocks. The findings were compatible with CNS Zika virus infection and concomitant bacterial meningitis and sepsis.

The placental tissues of case 4 showed dense and heterogeneous chorionic villi with calcification, sclerosis, oedema, increased perivillous fibrin deposition, and patchy lymphohistiocytic intervillitis (figure 3A). Immunohistochemical testing of placental tissue was positive for Zika virus, with antigens noted in Hofbauer cells in the chorionic villi (figure 3B). Zika virus RT-PCR was positive and sequence analysis showed 99% identity with Zika virus strains isolated from Brazil during 2015. RT-PCR assays were negative for dengue viruses.

Samples from case 5 showed predominantly endometrial tissue with Arias-Stella reaction. Minute fragments of placental tissue showed no significant findings. Immunohistochemical testing was negative for Zika virus. Zika virus RT-PCR was positive and sequence analyses showed 100% identity with Zika virus strains isolated from Brazil during 2015. RT-PCR assays were negative for dengue viruses.

**Discussion**

We report pathological findings of congenital Zika infection from two neonatal autopsies, one 2-month-old baby autopsy, and two spontaneous abortions with Zika virus infection that occurred in Brazil in 2015. Brain abnormalities included microcephaly, lissencephaly, cerebellar hypoplasia, and ventriculomegaly. Congenital CNS abnormalities have been previously associated with different infections including rubella virus, Coxsackie virus, toxoplasmosis, and enteroviruses.\textsuperscript{27,28} Histo-pathological assessment of the brain in the three fatal cases described in this report showed microcalcifications, scattered microglial nodules, cell degeneration, and necrosis. The antigens of Zika virus were localised in the cytoplasm of degenerating and necrotic neurons and glial cells. No immunohistochemical staining for Zika virus was identified outside of the CNS. The absence of a substantial inflammatory response in the brain and a specific cytopathic viral effect distinguishes Zika virus infection from other important viral infections that are
also associated with microcephaly and microcalcifications, such as cytomegalovirus and herpes simplex virus. The paucicellular inflammatory response in tissues is similar to what is seen in fatal congenital rubella syndrome.

The three fatal cases also showed a broad array of congenital malformations including congenital contractures, craniofacial malformations, craniosynostosis, pulmonary hypoplasia, and a wide range of brain abnormalities. These are seen in the syndrome known as fetal akinesia deformation sequence or severe arthrogryposis, which is caused by many disorders, including non-infectious and infectious agents such as enteroviruses and different TORCH pathogens (vertically transmitted) like Toxoplasma gondii, varicella-zoster virus, cytomegalovirus, rubella virus, and measles. The mechanism for these deformities in Zika virus infection are not entirely clear, but most probably result from neurotropism of the virus with subsequent damage of the brain and interference in neuromuscular signalling leading to fetal akinesia.

Our findings provide evidence that maternal Zika virus infection during the first trimester of pregnancy can result in placental and fetal damage and loss; however, additional case assessments are needed to further elucidate the pathogenesis of Zika virus and establish a correlation between transplacental infection and fetal brain injury. Placenta had villous oedema, increased numbers of Hofbauer cells, with Zika virus antigens seen in chorionic villi. Chronic histiocytic intervillositis was also seen; additional studies are needed to investigate whether this reaction is directly related to viral infection or represents a non-specific immune response. Pregnant women who reside in or have travelled to an area with ongoing Zika virus transmission and who report clinical illness consistent with Zika virus infection should undergo testing. Our findings suggest that assessment of fixed placental and fetal tissues and tissues from neonatal deaths with evidence of microcephaly can be useful to establish the diagnosis of congenital Zika infection. An important limitation of this report is the small number of cases assessed. Examination of additional cases of microcephaly, placentas, and embryonic tissues from spontaneous abortion is needed. Further investigation into the association between maternal Zika virus infection during the second or third trimester of pregnancy and adverse pregnancy outcomes, including assessment of placental and fetal tissue samples when available, will be important to identify appropriate measures to minimise the effect and assess the long-term sequelae of Zika virus infection.

Until now, there have been no reports of a mosquito-borne virus that could cause severe birth defects on a large scale. This report describes the detection of Zika virus viral RNA and antigens in brain tissues of fatal cases of congenital Zika infection and the further direct link between Zika virus infection and microcephaly, arthrogryposis, and spontaneous abortion. These findings also provide crucial insights into viral pathogenesis and tissue tropism.

The combination of clinical, epidemiological, and laboratory data is essential to confirm a diagnosis of Zika virus infection in fatal cases. Zika virus is an evolving epidemic, and many important questions remain to be explored. We are continuing to investigate the possible spectrum of neurological lesions in infants and various features of abnormalities in placental tissues. Analysis of FFPE tissues using the combination of histopathology, immunohistochemical assays, and RT-PCR improves Zika virus diagnosis, and can be particularly useful when fresh-frozen tissue and other conventional specimens are unavailable. Further assessment of tissue samples from pregnancy losses during different stages of fetal development will contribute to an understanding of the pathogenesis of congenital Zika infection and help to explore a mechanism for vertical transmission.

**Contributors**

RBM, JB, AMdeOR, HPFD, SD’AI, CTK, SR-S, YE, KGL, WHdO, and SRZ did the data collection. AMdeOR and HPFD did the autopsies for the two first cases and collected the tissue samples. RBM, JB, AMdeOR, HPFD, SD’AI, CTK, LS-F, MKK, GH, AM, JR, JG, DR, CSG, TS, W-JS, RI, AL, and SRZ did the laboratory analysis. RBM, JB, and SRZ wrote and edited initial drafts of the report. All authors did the data interpretation and reviewed the final draft.

**Declaration of interests**

We declare no competing interests.

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**Figure 3: Case 4—histopathological and immunohistochemical findings of the placenta**

(A) Low magnification of an 11-week placenta with massive chronic intervillositis and fibrin deposition; haematoxylin-eosin stain. (B) Zika virus immunolocalised in Hofbauer cells (arrowheads) and in association with karyorrhectic debris of the chorionic villi.
Zika and histopathology in first trimester infections

The Zika virus epidemic that started in Brazil has led to thousands of cases of devastating neuropathology and miscarriage, stillbirth, and neonatal death. Although the US Centers for Disease Control and Prevention has concluded that Zika virus causes microcephaly and other fetal brain defects, demonstration of Zika virus in tissues is sparse. In The Lancet, Roosecelis Brasil Martines and colleagues’ case series documents Zika viral infection and pathology in three autopsy cases and two spontaneous abortions and is the first study we are aware of linking Zika virus to congenital malformations. Samples from five cases, including two newborn babies with microcephaly and severe arthrogryposis who died shortly after birth, one 2-month-old baby, and two placentas from spontaneous abortions, from Brazil were assessed by pathological examination, immunohistochemical assays using a mouse anti-Zika virus antibody, and RT-PCR assays targeting the NS5 and envelope genes. Amplicons of RT-PCR positive cases were sequenced for characterisation of strains. The authors’ case series includes details necessary to formulate pathogenic hypotheses but prompts new mechanistic questions.

A major finding in Zika virus transmission is the relation between first trimester exposure and severe sequelae, similar to that seen for congenital rubella syndrome. Because maternal perfusion of the placenta does not occur until late first to second trimester, the mechanism of transplacental infection is not straightforward. Is there direct infection of the trophoblast (as shown in animal and in-vitro studies) that transmits virus to developing neural tissue? Martines and colleagues’ finding of Zika virus in early placenta supports a direct mechanism.

A placental pathological footprint of viral infection is crucial information providing diagnostic and causational data. Martines and colleagues describe three placentas, one from each trimester. The placental findings varied by trimester with virus identified in the first and second trimester placenta. Histopathological features were present only in the first trimester placenta. This 11-week gestational age placenta showed a specific pathological entity, chronic histiocytic intervillositis—a striking histopathology of unknown cause diagnosed by substantial intervillous histiocytosis (presumed to be maternally derived) admixed with intervillosus fibrin.

Rarely, intervillositis has been reported with infections—eg, malarial sequestration, acute psittacosis, and dengue virus infection. It is tempting to conclude that this chronic histiocytic intervillositis is related to Zika virus infection, but only one case is described in this report. Sherif Zaki—senior author of the report by Martines and colleagues—has seen other cases of Zika virus associated chronic histiocytic intervillositis, which may confirm the likely causation (Zaki S, personal communication). If placental histopathology is restricted to first trimester so-called acute infections, and then resolves, how? Can the virus infect the fetus without concurrent placental injury when infection occurs later in pregnancy, as happens with HIV and hepatitis C virus? More cases at various gestational ages will need to be pathologically studied.

Timing of infection is important to ascribe causality of the congenital malformations described. Three of the reported cases include examination of the brain, with early gestational infections: the first month of pregnancy (cases 2 and 3) or the first trimester (case 1). There was immunohistochemical and molecular evidence of virus persistence in the brain in these three cases. The range of neuropathology included ventriculomegaly, lissencephaly, which commonly aligns with microcephaly, and cerebellar hypoplasia—all of which have been observed in other cases studied. Brains also showed evidence of tissue destruction—calcifications, gliosis, and necrosis. The presence of
Martines and colleagues highlight the damage to the causal in perinatal complications and should include the full spectrum of congenital Zika syndrome. Additional detailed targets for Zika virus infection. It is possible that these findings indicate additional tissue necrosis suggests ongoing cellular injury, consistent with the demonstrated continued viral presence. Thus, the patterns of injury are likely to follow from both cellular injury at the time of infection as well as subsequent damage as the brain develops.

The evidence from cell culture systems places the neuronal precursor cell as a crucial target for Zika virus infection resulting in cell death. Loss of these cells early in development has been reported to substantially reduce the number of neurons generated and result in small brains without cortical gyration. The report by Martines and colleagues highlights the damage to the nervous system that can follow such infection—both from the primary injury as well as ongoing infection.

Additional questions arise from this report. Case 1, with maternal infection during the first trimester, also showed holoprosencephaly—a malformation resulting from failure of cleavage of the forebrain into the two secondary vesicles around week 5 of gestation, associated with alterations in SHH signalling, trisomy 13, and other putative teratogenic agents including infections. The timing of maternal infection must therefore be at this time period of development to result in holoprosencephaly. Craniosynostosis, premature fusion of the bones of the skull along suture lines, was also present in cases 1 and 2. Although this skull abnormality has been associated with holoprosencephaly in rare cases, it was noted independently in case 2. Because craniosynostosis reflects extracranial soft tissue development, independent of brain development, it is possible that these findings indicate additional tissue targets for Zika virus infection. Additional detailed pathological studies are needed to properly elucidate the full spectrum of congenital Zika syndrome.

Work remains to confirm Zika virus infection as causal in perinatal complications and should include histopathological examination of available tissues at various gestational ages. This report highlights that we can learn much about the pathogenesis of Zika virus congenital infection through careful pathological investigation, but leaves us with many questions for study.