However, hepatitis B vaccination during pregnancy remains an important prevention strategy that should be considered for pregnant women with HIV or other risk factors for hepatitis B for both maternal and fetal health.

Available research on maternal hepatitis A and B immunisation is scarce and more data are needed to determine the effectiveness of maternal hepatitis A or B immunisation to prevent neonatal infection. In the meantime, we support ACIP's recommendations that maternal immunisation be considered for high-risk women as part of a comprehensive strategy that includes universal hepatitis B testing during pregnancy, treatment for infected women and prophylaxis for infants when indicated, and universal infant immunisation to reduce the global burden of viral hepatitis.

We declare no competing interests.

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Multiple sites PCR testing for enteroviruses in young febrile infants

Jérémy Lafolie and colleagues[1] reported a prospective multicentre study that evaluated the incidence of enterovirus infection in neonates and young infants with suspected sepsis or fever without a source and children with suspected meningitis. Cerebrospinal fluid (CSF) and blood enterovirus PCRs were done between June 1, 2015, and Oct 31, 2015, and between June 1, 2016, and Oct 31, 2016. They demonstrated the increased diagnostic utility of blood PCR testing in addition to CSF testing, because blood PCR identified 24% of additional enterovirus cases that would have otherwise been missed. In the accompanying Comment, Kevin Messacar and Samuel Dominguez[2] suggested the possibility of also testing samples obtained from non-sterile sites and focusing on periods of higher enterovirus circulation.

At Nationwide Children's Hospital (Columbus, OH, USA), enterovirus PCR testing has been routinely done using blood, CSF, and samples from superficial non-sterile sites (including mucosa, such as throat, eye, and rectum, and skin lesions) in all patients younger than 60 days hospitalised for fever or presumed sepsis, since 2013. We did a retrospective review of infants who had positive enterovirus PCR results from any site between January, 2015, and September, 2016. During this period, 783 patients were tested, and 144 (18%) had a positive enterovirus PCR result. Similar to Lafolie and colleagues' findings,[1] the numbers of enterovirus cases detected were larger between the months of June and September (figure). However, we also identified enterovirus from clinical samples in all but 3 months of our study period. Of the 144 positive enterovirus PCR results, 34 (24%) were identified between the months of October and May. The seasonality also varied between study years (figure). Enterovirus PCR was positive in 75 (52%) infants in CSF samples, 103 (72%) in blood samples, and 119 (83%) in samples from non-sterile sites. 51 (35%) infants had positive results from all three sites, another 51 infants had positive results from two sites, and 42 (29%) infants had enterovirus-positive results in only one of the three sites tested. Among those with only one positive site, the majority were positive in samples from non-sterile sites—27 (19%) of 144—followed by blood samples in 13 (9%), and in CSF samples in two (1%). Infants' median age was 24 days (IQR 15–35); all were full term with normal birthweight, and 50% were

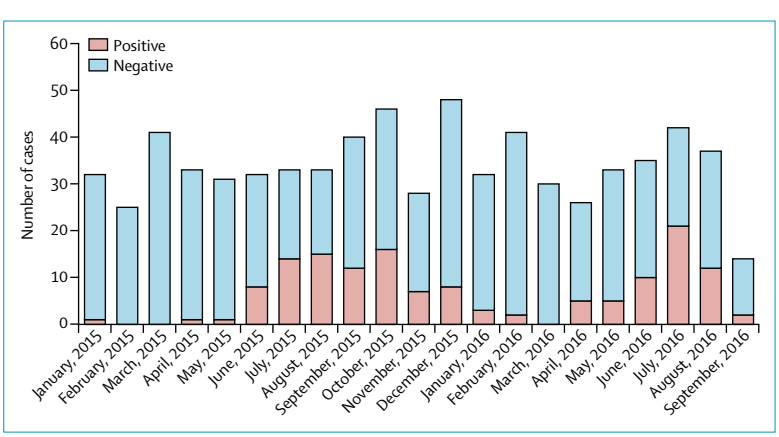

Figure: **Monthly distribution of positive enterovirus PCR from cerebrospinal fluid, blood, and non-sterile sites in infants younger than 60 days**

boys. Most common symptoms were fever (94%), respiratory symptoms (17%), rash (16%), and diarrhoea (8%). Four infants (3%) had seizures, and two required inotropic support. Six (4%) infants were admitted to the paediatric intensive care unit. 16 (11%) infants had leucopenia, and 59 (41%) had lymphopenia. Of the infants (n=75) with positive CSF PCR results, the median CSF white blood cell count was 50 cells per µL (IQR 5–343); a third of these infants had no CSF pleocytosis. In addition to qualitative PCR results, we also measured semi-quantitative viral loads (C_t values) and found that enterovirus loads were higher (lower C_t values) in blood (Ct median 32·30; 29·91–36·42) than in both CSF (35·88; 34·02–37·51); p<0·0001) and non-sterile site (34·72; 32·02–37·35; p=0·0014) samples. The median duration of hospitalisation was 41 h (IQR 34·11–48·23). No deaths or reported complications at discharge occurred.

Enterovirus infection should be considered in the diagnosis of febrile infants, and testing multiple samples, including those from non-sterile sites, could increase the diagnostic yield and decrease unnecessary health-care costs.[1–4] We suggest paediatric centres consider implementing routine, year-round testing for enterovirus. At Nationwide Children's Hospital, routine testing of infants for enterovirus not only gave us a better understanding of viral epidemiology but also improved the clinicians' management strategies, leading to shortened duration of antimicrobial therapy and hospitalisation.

We declare no competing interests.

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The difficulties in obtaining reliable Zika virus diagnostics

We read the Personal View by Marion Koopmans and colleagues[1] with great interest. The authors highlight two major obstacles delaying the international response to the catastrophic Zika virus outbreak that has struck Brazil and is ongoing in different parts of Latin America. First, the delays in obtaining dedicated funding. Second, the difficulties of ensuring adequate laboratory diagnostics in Latin America, which the authors illustrate with the inability to establish an external quality assurance for Zika virus detection within the EU-funded ZIKAlliance project.[1]

As ZIKAlliance partners, we fully agree with these challenges. Notably, we can provide crucial insight into laboratory quality in affected regions. In 2017, we did a Zika virus external quality assurance in Brazil. 73% of Brazilian laboratories presented with reduced sensitivity and specificity in detection of Zika virus genetic material.[2] The overall risk of false-negative results was 16·7% (95% CI 5·4–28·0), and the overall risk of false-positive results was 26·7% (5·0–48·5). The high risk of incorrect test results is alarming, but not restricted to resource-limited areas, such as Brazil. Albeit to a lesser extent, similar problems were also observed in European laboratories, for which the overall risk of false-negative results was 14·5% (8·9–20·1) and the overall risk of false-positive results was 4·4% (2·1–6·8).[3] The similarities between the studies from Brazil and Europe show that adequate laboratory diagnosis of Zika virus infection is universally challenging. Unreliable Zika virus diagnostics have a huge effect on individual and public health, potentially including illegal abortions based on false-positive test results,[4] unwarranted delays of pregnancy, and biased estimates of the absolute risk of congenital disease upon maternal infection during pregnancy.[2]

The article we published on our study[2] attracted broad attention. After our paper went to press in May, 2018, the largest Brazilian newspaper, *Folha de São Paulo*, reported on our results.[5] Subsequently, we were approached by Brazilian stakeholders to reveal the identity of our study participants. We were unable to comply with this request since confidentiality is crucial to ensure participation of laboratories in external quality-control studies, particularly in resource-limited settings such as Brazil.

Access of laboratories in affected regions to state-of-the-art reagents and external quality control is an unresolved key component of outbreak response.[2] Thus, national and supranational stakeholders must support public laboratories in outbreak regions that commonly deal with a huge burden of testing in the absence of adequate reagent supply.

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