

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 (C_t 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 colleagues¹ 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 study² 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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An update on the severe outbreak of HIV in Kot Imrana, Pakistan

Kot Imrana (Kot Momin) is a small village located in the district of Sargodha in Punjab province, Pakistan. During PCR-based screening by The Health Department at District Headquarter Hospital, Sargodha, in January 2019, 669 (13.38%) of the 5000 people who live in the village were found to be HIV-positive. In this journal, Muhammad Zaid and Muhammad Sohail Afzal¹ reported that the prevalence of HIV in the same village in June, 2018, was 1.29%. The HIV epidemic in Kot Imrana, therefore, continues to grow at an alarming rate with a substantially high mortality. Upon interviewing patients, a quack was found to have used the same syringe on multiple patients, and he himself died of AIDS later in 2018, leaving hundreds of patients HIV-positive. Prevalence was found to be higher in women and children. The detailed interviews of patients who visit our laboratory confirmed that more than 96% of HIV-positive patients were unaware of the modes of transmission of HIV and the

consequences of this life-threatening virus. The first case of HIV was reported in Kot Imrana around 15 years ago and now it is the most heavily affected region of Pakistan (a country in which more than 110 million people are at risk of infection), largely because it is located in Punjab, the largest and the most densely populated province of the country.

According to AIDS control programme data,² 5000 quacks working in Sargodha are transmitting the virus to the community. Additionally, barbers are the other source of HIV transmission because they use contaminated razors and blades. A substantial proportion of patients might have acquired the infection sexually, but they were reluctant in discussing their sexual relationships. According to local resources, the virus has been spread to nearby villages and about 900 HIV carriers are living in Sargodha. Discrimination and stigma associated with HIV/AIDS is another risk factor that makes elimination of HIV a challenge in socially conservative settings, such as Pakistan and other developing and under-developed countries.

Laboratory data from Genome Centre for Molecular Based Diagnostics and Research, Lahore, Pakistan, showed an increased prevalence of HIV in the Mardan region of the Khyber Pakhtunkhwa province. From this trend, we can conclude that Sheikhpura, Lahore, Faisalabad, and more than 50 villages in the vicinity of Sargodha, as well as more than 50 rural and urban areas in the outskirts of Mardan, are potential hotspots of HIV infection in Pakistan.

Scenarios suggest that the overall prevalence of HIV in the general population is underreported in Pakistan. The National AIDS Control Programme of the Government of Pakistan must develop innovative strategies and launch awareness campaigns to control HIV through increased access to screening, treatment, and counselling. This

HIV epidemic in Kot Imrana is unprecedented, having expanded from a prevalence of 1.29% to one of 13.38% in just 6 months. Focus on this high transmission area is urgently needed for the proper implementation of evidence-based prevention strategies to control the epidemic and reduce the transmission of virus.

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Yellow fever: is Asia prepared for an epidemic?

Epidemic arboviral diseases are relentlessly increasing in incidence, fueled by urbanisation, scarcity of effective disease and vector control strategies, and globalisation resulting in disease exportation.¹ This decade, the number of travellers exporting yellow fever virus to non-endemic countries is at a record high.² Furthermore, in 2016, for the first time in documented history, confirmed yellow fever virus was exported in travellers from Africa to Asia, where about 2 billion immunologically naive people live in areas inhabited by the *Aedes aegypti* mosquito vector and are at risk for transmission.³ The case-fatality rate of yellow fever is among the highest of all arboviral diseases, underscoring the threat of a newly emerging epidemic disease problem in Asia.

Why outbreaks of yellow fever have not yet occurred in Asia is unknown. The factors influencing transmission dynamics of yellow fever (eg, vector competence and magnitude and