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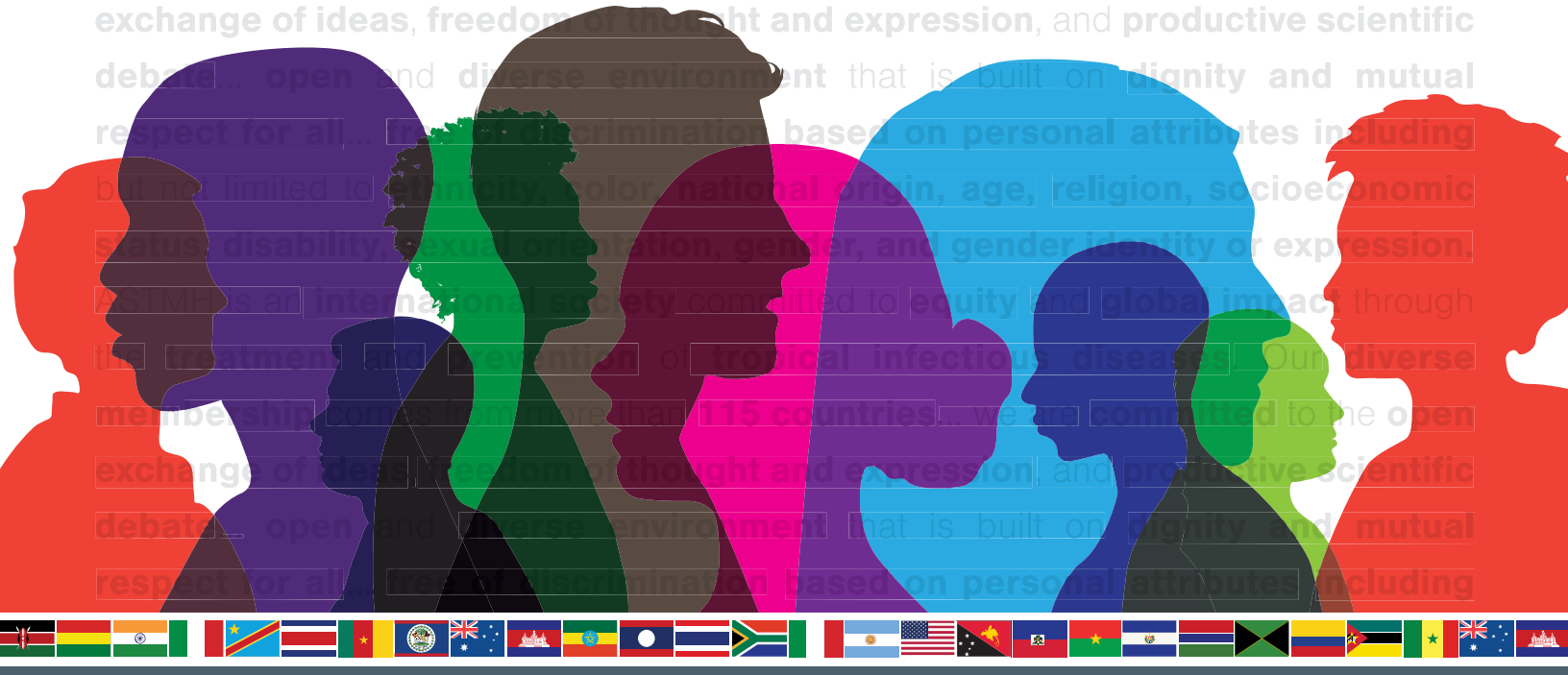
ABSTRACT BOOK



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eliminated virus infectivity but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants for use in vaccine design. To identify uncharacterized DENV cellular receptors we assayed wild-type DENV RVP infectivity in non-permissive cells expressing our membrane proteome array (MPA) of 5,300 unique human membrane proteins. This has identified candidate membrane proteins that enable DENV infectivity. We have identified neutralizing epitopes in DENV prM/E and specific sites that are critical for DENV infectivity, providing new targets and opportunities for vaccine development.

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INTERCURRENT FLAVIVIRAL VIREMIA IN ILL RETURNED TRAVELERS WITH *PLASMODIUM VIVAX* MALARIA

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Similar epidemiology and clinical presentations of arboviral infections and malaria coupled with the typically sequential approach to diagnostic testing, where malaria is confirmed or excluded urgently in febrile returned travelers, may mask the true epidemiology of co-infections. Flaviviruses are known to trigger relapsing forms of malaria, including *Plasmodium vivax*, long after primary malaria infection, and this may delay the diagnosis of malaria. We aim to understand the incidence of intercurrent flaviviral infection in confirmed *Plasmodium vivax* infection. DNA and RNA from biobanked isolates of *P. vivax* detected in whole blood at the Public Health Ontario Laboratory between 2006 and 2018 were extracted and screened for intercurrent flaviviral infections using previously validated real-time PCR (qPCR) assays targeting multiple flaviviruses (pan-FLAV) and, specifically, dengue virus types 1-4 (DEN1, DEN2, DEN3, DEN4). Five hundred and two unique isolates of *P. vivax* were identified, of which 90 have been tested to date. Males accounted for 65.6% (n=59/90) of *P. vivax* cases, while females accounted for 32.2% (n=29/90), and sex was unassigned in 2.2% (2/90). Median age of *P. vivax* cases was 33.2 years (range 3.7 years - 85.8 years; IQR 23.4 - 45.9 years). Median parasitemia was 0.2% (range < 0.01% - 1.1%). Fifty-nine (65.6%) *P. vivax* cases had documented travel history exclusively to South Asia, with India as the most common source country (22/90 [16.7%]). Pan-FLAV assay yielded a 1.1% (1/90) positivity rate. Pan-DEN assay will be performed next. Intercurrent flaviviral viremia was noted in at least 1.1%, which may suggest that primary flaviviral infection triggered a relapse of *P. vivax*. Alternatively, such co-occurrence may suggest primary infection with both organisms known to cause fever in returning travelers. Consideration of flaviviral co-infection should be given to the *P. vivax* patient with deep thrombocytopenia, lymphopenia, and high-yield arboviral symptomatology such as rash and retro-orbital headache.

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DETECTION AND SEQUENCING OF ZIKA VIRUS IN NORMOCEPHALIC NEWBORNS WITH CONGENITAL ZIKA IN FECTION

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In 2015, Brazil has experienced an unprecedented Zika virus (ZIKV) outbreak and later that year, an unexpected outbreak of newborns with microcephaly occurred in major cities in northeastern Brazil, associated with Congenital Zika Infection (CZI). Most descriptions and publications regarding CZI focus on the clinical presentation of newborns and infants with microcephaly. Scarce information is available concerning CZI without microcephaly. During hospital surveillance for CZI in a reference maternity hospital, we identified 14 normocephalic newborns with confirmed CZI. Eight (57%) of the newborns were female and the mean gestational age at birth was 38.46 ± 1.90 weeks. The mean of head circumference was 38.57 ± 1.40cm. The transfontanel ultrasonography was performed in 13 (92.9%), and no alterations were observed in any of the cases. All newborns had a positive RT-PCR confirming the diagnosis of CZI, mostly in urine samples (57%). In two of the cases, ZIKV were detected in 2 distinct samples. ZIKV-specific RT-PCR amplification products have been obtained and NS5 gene fragments (426-bp) were obtained using Sanger sequencing. The phylogenetic analysis showed that the isolate belongs to the Asian genotype and clusters closely with strong bootstrap support (>90%) with sequences isolated in Northeast and Northern regions of Brazil. With this, we infer that CZI could present in a broad spectrum of clinical manifestation, including the asymptomatic presentation at birth. It is necessary careful surveillance to identify cases with few or no symptoms at birth and a close follow-up for early detection of clinical manifestations of CZI and timely intervention.

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ZIKA VIRUS DETECTION IN PREVIOUSLY UNDIAGNOSABLE SAMPLES: OPTIMIZATION OF A QUANTITATIVE RT-PCR ASSAY FOR SAMPLES OF LOW VIRAL CONCENTRATION

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The Zika virus (ZIKV) outbreak of 2015 affected many countries in Latin America and highlighted a pressing need for sensitive molecular diagnostics to detect the presence of ZIKV in various sample types. At the time, only two RT-qPCR assays, developed by Faye et al. and Lanciotti et al., were available to diagnose human ZIKV infections. These assays used primer sets that target separate regions of the ZIKV genome with enough specificity to serve as diagnostic tools that could differentiate ZIKV from other related arboviruses, such as dengue and yellow fever. However, their sensitivity was low, resulting in false-negative diagnosis of many ZIKV cases, particularly when ZIKV was present at low levels (CT values around 30 or higher). We developed an improved RT-qPCR protocol that uses optimized primer sets and cycling conditions to detect the presence of ZIKV at levels previously considered undiagnosable (CT values up to 38). The parameters optimized include primer length, GC content and annealing temperature. We also created a positive control plasmid that includes both the NS5 and envelope regions of the genome, allowing for side-by-side comparisons of the amplification efficiency of old and new assays. Using this control plasmid, we estimated the limit of detection of our new assay at 31 copies of the viral genome. We used our assay to test a number of samples suspected of being false-negatives and found that about 10% of those re-tested were positive for ZIKV. Our results indicate that the improved ZIKV assay can help address the issue of false-negatives during ZIKV diagnosis and will be a useful tool for those interested in a fast, rapid, and sensitive diagnostic for ZIKV, particularly in samples of low viral concentration encountered during clinical evaluation or research.