Development and evaluation of a viral-specific random PCR and next-generation sequencing based assay for detection and sequencing of hand, foot, and mouth disease pathogens

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Background: Hand, foot, and mouth disease (HFMD) has become a major public health problem across the Asia-Pacific region, and is commonly caused by Enterovirus A, including enterovirus A71 (EV-A71) and coxsackievirus A (CV-A) 6, 10 and 16. Generating pathogen whole-genome sequences is essential for understanding their genetic diversity and phylodynamics. The frequent replacements among serotypes of Enterovirus A and a limited numbers of whole-genome sequences available in GenBank hinder the development of overlapping PCRs for whole-genome sequencing.

Methods & Materials: We developed and evaluated a viral-specific random PCR (rPCR) and next-generation sequencing based assay for sequence-independent whole-genome amplification and sequencing of HFMD pathogens. A total of 14 EV-A71/CV-A6/CV-A10/CV-A16 PCR positive rectal/throat swabs (Cp values: 20.9 – 33.3) were used for assay evaluation.

Results: Our viral-specific rPCR evidently outperformed the normal rPCR in terms of the total number of EV-A71 reads and the percentage of EV-A71 reads: 3% vs. 0.1% for the sample with Cp value of 30 and 6% vs. 0.91% for the sample with Cp value of 26, respectively. Additionally the assay could generate genome sequences with the percentages of coverage of 94%-100% of 4 different HFMD causing enteroviruses in 73% of the tested rectal/throat swabs, representing the first whole-genome sequences of CV-A6, CV-A10 and CV-A16 from Vietnam, and could assign correct serotyping results in 100% of the tested specimens. In all but one the obtained consensuses of two replicates from the same sample were 100% identical, suggesting that our assay is highly reproducible.

Phylogenetic analysis of the obtained sequences in this study suggested that the EV-A71 strains sampled in 2012 belonged to subgenogroup C4, whereas the viruses collected in 2013 belonged to subgenogroup B5. All CV-A16 sequences belonged to genogroup B1a, and showed a close relatedness to the viruses circulating in the Asia-Pacific region. Meanwhile the CV-A6 and CV-A10 strains were closely related to the corresponding HFMD-causing viruses from various parts of the world including Europe and Asia.

Conclusion: In conclusion, we have successfully developed a viral specific rPCR and next-generation sequencing based assay for sensitive detection and direct whole-genome sequencing of HFMD pathogens from clinical samples.

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in Romania, has higher abundant populations and its distribution extended over all the risk areas. Anopheles daceae, possible malaria vector, has an extended distribution and higher densities than Anopheles messae everywhere.

**Conclusion:** The malaria re-emergence risk maintains in Romania in conditions of the climate and other environmental changes. There is the need of the permanent surveillance of the factors influencing this risk to prevent and control malaria re-appearance.

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Evidence of presence of antibodies against selected arboviruses in Ijara and Marigat Districts, Kenya

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**Background:** Arboviruses are transmitted by arthropods with humans becoming infected during blood feeding by infected mosquitoes, ticks and sandflies. Characterization of arbovirus circulation and transmission in industrialized countries has been well documented, but there are many knowledge gaps in developing nations. Entomological surveys conducted so far have indicated circulation of arboviruses of significant public health importance in Aedes, Anopheles and Culex species in vast populations in Kenya, suggesting the presence of competent vector systems.

**Methods & Materials:** The human involvement in the transmission cycle of these viruses has, however, not been demonstrated. This study sought to determine the circulation of a range of arboviruses including Chikungunya, Dengue, Sindbis, Sandfly Naples, Sandfly Sicilian, Uganda S, West Nile and Zika viruses in Ijara and Marigat Districts where vector surveillance has been done.

**Results:** A total of 351 patient serum samples were analyzed for presence of antibodies using IgG ELISA. Of these, 190 (54.2%) were female and 161 (45.8%) were female, with ages ranging between 1 and 73. These were hospital based patients who presented to the hospital with fever of unknown origin. The overall arbovirus percentage circulation among these patients was 53/351 (15.1%) with 7% (10/143) in Marigat and 21% (43/208) in Ijara. Of the positives, flaviviruses were 68%, alpha viruses 29.6% and bunyaviruses 1.4%. Uganda S Virus was the highest in circulation at 10%, followed by West Nile virus 6%, Sindbis 5%, Dengue 2%, Chikungunya 1.1%, Sandfly Naples 0.2% respectively. Semliki-forest virus-specific antibodies were detected by plaque reduction neutralization test in 3/351 (0.85%) persons tested. Antibodies against Sandfly Sicilian and Zika viruses were not detected. This study constitutes the first detection of antibodies against Sandfly Naples virus in Kenya.

**Conclusion:** The study has demonstrated the presence of antibodies against selected arboviruses in the two sites amongst the