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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 consensus 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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Could malaria re-emerge in Romania?

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Background: Romania was an endemic malaria country with as many as 300,000 new cases yearly and the eradication was completed in 1962. The permanent risk of malaria re-emergence in Romania maintains because of the simultaneous presence of the Anopheles maculipennis group vector species and imported malaria cases. The risk increased in the present conditions of climatic and other environmental changes.

Methods & Materials: The analysis of the historical and present data regarding the presence, abundance and distribution of different vector species in Romania in correlation with the environmental, social and economic conditions led to the evaluation of the risk evolution of malaria re-appearance and the main factors involved. The entomological data were integrated with earth observation data obtained by spatial technologies and mapped to put in evidence the stratification of the present risk areas of malaria re-emergence.

Results: The general level of malaria re-emergence risk varied in Romania. The risk was low after eradication linked to the low densities of vector populations because of the climatic conditions maintained in usual limits and intensive agriculture on large uniform areas. The risk gradually increased after 1990 linked to the high abundance of vector populations as before malaria eradication because of the global climate change and the land use change in Romania leading to the fragmentation of the habitat in the agricultural areas produced by the resuming to the traditional work on small private pieces of land.

The present risk areas of malaria re-emergence are mapped. They generally overlap the former malaria stratification areas in Romania in accordance to the distribution of different vector species in the landscape. The new aspects are linked to the present environmental changes. Anopheles atroparvus, the main vector