Type: Poster Presentation

Final Abstract Number: 41.248
Session: Poster Session I
Date: Thursday, March 3, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

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

A.T. Nguyen[1,*], N. Nghiem[2], T. Tran[1], V. Hoang[3], N. Le[1], Q. Phan[2], N. Le[4], V. Ho[3], V. Do[3], T. Ha[3], H. Nguyen[4], C. Nguyen Van Vinh[2], G. Thwaites[1], H.R. van Doorn[5], T. Le[1]

[1] *Oxford University Clinical Research Unit, Ho Chi Minh, Viet Nam*
[2] *Hospital for Tropical Disease, Ho Chi Minh, Viet Nam*
[3] *Children's Hospital 2, Ho Chi Minh, Viet Nam*
[4] *Children's Hospital 1, Ho Chi Minh, Viet Nam*
[5] *Oxford University, Ho Chi Minh City, Viet Nam*

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.

http://dx.doi.org/10.1016/j.ijid.2016.02.435

Type: Poster Presentation

Final Abstract Number: 41.249
Session: Poster Session I
Date: Thursday, March 3, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Could malaria re-emerge in Romania?

G. Nicolescu[1,*], V. Purcarea-Ciulacu[1], A. Vladimirescu[1], G. Dumitrescu[2], D. Saizu[3], E. Savin[3], I. Sandric[3], F. Mihai[3]

[1] *Cantacuzino National Institute of Research, Bucharest, Romania*
[2] *Army Centre of Medical Research, Bucharest, Romania*
[3] *S.C. Esri Romania S.R.L., Bucharest, Romania*

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

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

http://dx.doi.org/10.1016/j.ijid.2016.02.436

Type: Poster Presentation

Final Abstract Number: 41.250
Session: Poster Session I
Date: Thursday, March 3, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Co-occurrence of mosquito larval in natural and artificial habitats in Mazandaran Province, northern Iran

S.H. Nikookar*, A.-A. Enayati, S.P. Ziapour, S.N. Mousavi Nasab, M. Fazeli-Dinan, J. Shojaee

Mazandaran University of Medical Sciences, Sari, Iran, Islamic Republic of

Background: Different species of mosquitoes may have a role in the transmission of various diseases. The presence of more than one species in a habitat suggests that they share similar environmental conditions. Notwithstanding the importance of mosquitoes in the potential transmission of disease and intra and inter-species association, yet accurate data in relation to ecology and co-occurrence of mosquito species in the habitats is not available. Therefore, determining co-occurrence of this species in different habitats can be important to provide basic information in relation to vector control programs in the region. The present study focused on incidence of co-occurrence among mosquito species in Mazandaran Province, northern Iran.

Methods & Materials: Larvae collections were carried out from natural and artificial different habitats by standard dipping (350 cc) methods during May to December 2014, in 30 rural of 16 counties. Larvae collected from each larval habitats individually were preserved in test tubes containing lactophenol and sent to the laboratory for identification by valid key.

Results: Larvae were sampled from 120 habitats and identified by morphological keys. Sixteen species of mosquitoes were identified: *An. claviger, An. hyrcanus, An. maculipennis, An. marteri, An. plumbeus, An. pseudopictus, Cx. pipiens, Cx. tritaeniorhynchus, Cx. torrentium, Cx. perexiguus, Cx. territans, Cx. mimeticus, Cx. hortensis, Cs. annulata, Cs. longiareolata*, and *Cs. morsitans*. In total, larvae were seen in 1305 co-occurrences in natural and artificial oviposition sites during the study. The highest co-occurrence was observed associated with *Cx. pipiens* (630 occurrences, 48.27% of the total), *An. maculipennis* (87 occurrences, 6.66% of the total), respectively. Of these, *Cx. pipiens* was found in 123 occasions in related to *Cx. torrentium* which demonstrates the highest co-occurrences of the species. *An. marteri* (1, 0.07% of the total) and *Cs. morsitans* (2, 0.15% of the total) indicated the lowest occurrence in the province.

Conclusion: *Cx. pipiens*/*Cx. torrentium* shows highest co-occurrence in larval habitats in the province**.** **C**o-occurrence strengthen the common needs of these two species in the area which could indicate necessity further studies related to the bionomics *Cx. pipiens*/*Cx. torrentium* species, to provide adequate and affordable basic data for control programs in the future in the province

http://dx.doi.org/10.1016/j.ijid.2016.02.437

Type: Poster Presentation

Final Abstract Number: 41.251
Session: Poster Session I
Date: Thursday, March 3, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

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

V.O. Ofula[1,*], J. Oundo[2], Z. Irura[3], E. Chepkorir[4], C. Tigoi[5], J. Ongus[6], R. Coldren[7], R. Sang[5], R. Schoepp[8], C. Rossi[8]

[1] *USAMRD-K, Nairobi, Kenya, Kenya*
[2] *USAMRD-K, Nairobi, Kenya*
[3] *Ministry of Public Helath and Sanitation, Nairobi, Kenya*
[4] *International Center for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya*
[5] *International Centre of Insect Physiology and Ecology, Nairobi, Kenya*
[6] *Jomo Kenyatta University of agriculture and technology, Nairobi, Kenya*
[7] *USAMRU-K, Nairobi, Kenya*
[8] *USAMRIID, Washington DC, USA*

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 69%, 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

based assay for detection and sequencing of. hand, foot, and mouth disease pathogens. A.T. Nguyen1,âˆ—, N. Nghiem2, T. Tran1, V. Hoang3Â speciï¬c random PCR (rPCR) and next-generation sequencing based. assay for sequence-independent whole-genome ampliï¬cation 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 â€“. 33.3) were used for assay evaluation. Results: Our viral-speciï¬c 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. Next generation sequencing (NGS). Two cell lines DNA, NCI-H157 and A549, were used for the optimization of NGS library preparation, including different PCR primer pairs and number of PCR cycle.Â The ddPCR limit of detection for some assays is sometimes compromised by poor discrimination of the end point signal from other clusters in a two-dimensional histogram, which can lead to false positives.Â Based on of the effects of sequencing depth on the noise level, two different levels of sequencing depths with an average of 43,000 and 196,000Ã— were performed to measure the background (Table 2). A T test revealed that there was no significant difference in noise level for all 7 mutations between the two different depth levels and the two library. A52 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. Virus Evol. 2017 Mar 5;3(Suppl 1):vew036.051. doi: 10.1093/ve/vew036.051. eCollection 2017 Mar. Authors. Nguyen To Anh 1 , Tran Tan Thanh 1 , Hoang Minh Tu Van 2 , Nghiem My Ngoc 3 , Le Nguyen Truc Nhu 1 , Le Thi My Thanh 3 , Phan Tu Qui 3 , Truong Huu Khanh 4 , Le Nguyen Thanh Nhan 4 , Ho Lu Viet 2 , Do Chau Viet 2 , Ha Manh Tuan 2 , Nguyen Thanh Hung 4 , Nguyen Van Vinh Chau 3 , Guy Thwaites. 1 , H Rogier van Doorn 1 , Le Van Tan 1. Affiliations. 1 Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam.