

GloPID-R report on chikungunya, o'nyong-nyong and Mayaro virus, part 5: entomological aspects

Supplemental data - Detailed expert recommendations

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ALL THREE VIRUSES

A. Vector competence studies

To allow comparison between results from different laboratories, protocols for vector competence studies should be standardized. In the first line, the number of mosquitoes collected in the field to generate the lab colony for infections is critical as the number of progenitors collected reflects the genetic variability of the sample. To avoid colonization effect on VC results, low generations (<5) in controlled conditions of insectaries are advisable. In the same vein, low viral passages are preferred to old viral strains. Attention should be also paid to the preparation of the infectious blood meal: viral titer used, nature of viral stocks (cell type for viral production), nature of blood (animal/human, washed/unwashed). Other parameters can also introduce variations in results: temperature of incubation, duration of blood feeding, days post-infection for mosquito examination, and parameters measured. In the second line, the mosquito microbiome (gut bacteria, inherited endosymbionts, and insect specific viruses) can have diverse effects on host biology, including susceptibility to pathogens such as arboviruses in mosquitoes. So, mosquito microbiota can be the source of uncontrolled experimental variation in vector competence experiments between different labs. It is advisable to include a mosquito colony in all experimental infections; this colony should be produced from a field collected population and changes in the mosquito microbiome should be tracked to determine the effects of colonization in the insectary on microbiome (bacterial and viral). Using this control in all experiments would give an idea of the deviation from natural populations.

B. Experimental evolution studies

Experimental selection with multiple passages of viruses in mosquitoes may prove useful. Each passage will be comprised of: (i) oral infection of mosquitoes, (ii) collection of mosquito saliva, (iii) amplification of virus by inoculating collected saliva onto cells, (iv) collection of supernatant containing newly produced viruses, and (v) initiation of the next passage with oral infection of mosquitoes. Examination of viral populations at each passage will allow detecting genetically fixed variants.

C. Surveillance of sylvatic reservoirs and emergence events

Changes in mosquito distribution often lead to circulation of pathogens they transmit. The circulation of MAYV in endemic or enzootic regions in America, and CHIKV and ONNV in different regions in Africa should be monitored, in order to assess the risk of emergence in human and/or animal populations.

D. Vector control

A major effort is necessary to design adapted vector control strategies. The use of endosymbionts such as *Wolbachia*, genetically modified mosquitoes with refractory phenotype to arboviruses, sterile mosquitoes are as much possibilities to envisage on the field.

E. Molecular determinants of replication in vectors and transmission

To investigate more deeply interactions between mosquitoes and arboviruses, development of molecular tools, reverse genetics methods, genome sequencing of more mosquito species and improving mosquito reference genomes are urgently required.

CHIKUNGUNYA VIRUS

A. Vector competence studies – Europe

The fact that the environment can strongly modify adaptive properties of genotypes should encourage to develop more experimental assessments of vector susceptibility using field-collecting mosquitoes submitted to different environmental temperatures.

B. Vector competence studies – Africa (I)

Improvements in collection methods and rearing procedures should be encouraged to augment studies using local *Aedes* mosquitoes, in particular *Aedes aegypti formosus*.

C. Vector competence studies – Africa (II)

To better understand the distribution of *Ae. aegypti aegypti* vs *Ae. aegypti formosus*, more collaborations between the different regions of Africa should be fostered and notably between West/Central Africa and East Africa.

D. Vector competence studies – Pacific region

More studies should be promoted by local research agencies to better design control strategies against local *Aedes* species. Likewise, funding sources can be dedicated to more knowledge on their genome sequences, helping in a better understanding of their evolutionary biology in these fragmented islands, considered as hot spots of endemism.

E. Vector competence studies – Americas

To better understand vector competence of zoophilic mosquitoes, collection methods and mosquito rearing are still to be improved for these species less adapted to colonisation in insectaries. This gap is to be filled to measure the risk of potential spill back of CHIKV into a sylvatic cycle as did YFV in the past.

F. Co-infections in mosquitoes

Improving our understanding of the role of virome/microbiome in mosquitoes' ability to transmit co-infecting pathogens is necessary. Collaborations to obtain financing for the local teams and capacity-building research activities, access and development of research facilities for experimental infections, should be encouraged by national and international research funding agencies. Moreover, since co-

infections are likely in vectors (as DENV-CHIKV co-infection reported in *Aedes* mosquitoes [1]), mass viral screening should be facilitated with the development of multiplex approaches and high-throughput systems. These technologies will provide an exhaustive inventory of arboviruses carried by mosquitoes using the same batches of field collected mosquitoes for nearly 100 viruses screened.

O'NYONG-NYONG VIRUS

A. Vector competence studies

To investigate ONNV competence of *Anopheles* and *Aedes* species, knowledge of antiviral barriers in *Anopheles* and viral persistence in *Aedes* should be investigated. Development of molecular tools (reverse genetics studies for infectious/chimeric clones), experimental evolutionary studies may help to identify and dissect the molecular mechanisms that permit virus infection in *Aedes* and virus exclusion in *Anopheles*.

B. Enzootic cycle

Ecological and epidemiological studies to identify and characterize the putative ONNV enzootic cycle should be encouraged, in order to be prepared for future emergences in the image of what happened for ZIKV qualified as harmless before the 2015 pandemic.

MAYARO VIRUS

A. Vector competence studies (I)

To better understand the role of *Haemagogus*, *Sabethes* and *Aedes* species in MAYV transmission, viral isolations from field-collected mosquitoes and vector competence studies should be promoted. This would help to assess the potential of MAYV to cause millions human cases like CHIKV and ZIKV did in a recent past. Likewise, molecular tools (e.g. infectious clones) to examine viral molecular determinants for mosquito susceptibility are advisable.

B. Vector competence studies (II)

Vector competence of mosquitoes from African continent should be assessed for MAYV, in order to evaluate the risk of possible wider spread.

C. Potential urban cycle

Contrary to CHIKV, MAYV makes attempts to jump outside the enzootic cycle in the Amazon basin with a growing number of human cases reported. Change of vector host range should be tracked.

- [1] Caron M, Paupy C, Grard G, et al. Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. *Clin Infect Dis* 2012; 55: e45-53.