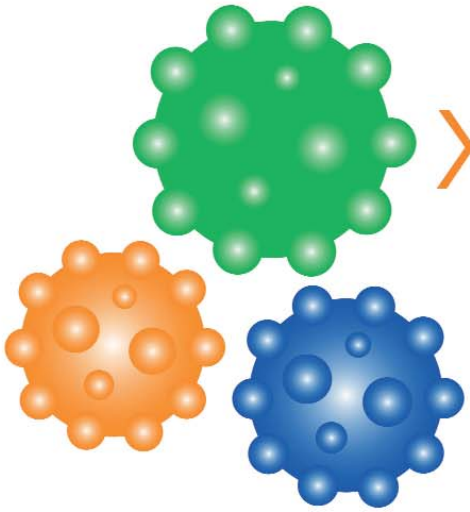

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Broad Molecular Detection of Flaviviruses

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Objectives: To provide an easy, simple and rapid assay for general molecular detection of flaviviruses.

Methods: The PCR assay presented here is developed using similar techniques as previously described (1). Primers were designed from an alignment containing 1159 flavivirus NS5 gene sequences. Two PCRs are involved in a semi-nested format. The first PCR (pre-amplification) include a single degenerate primer pair. The second PCR include 19 tagged primers at low concentration and one labeled detection primer corresponding to the tag-sequence. The reverse primer is the same for both steps. RNA from isolates of West Nile Virus (WNV), Yellow Fever Virus (YFV), Japanese Encephalitis Virus (JEV), Dengue Viruses (DENV1-4) and Tick borne Encephalitis Virus (TBEV) as well as the Usutu virus and Bagaza virus were used to evaluate the broad detection capacity of the assay. In addition, 65 clinical samples were used to evaluate the assay. They included human sera and urine, brain samples from birds and horses and mosquito and tick vectors.

Results: All isolates were successfully detected. The analytical sensitivity of the assay was evaluated using two different panels (WNV and DENV) from the Quality Control in Molecular Diagnostics organization (QCMD; http://www.qcmd.org/) from 2011. The detection limit was 104 copies/ml of WNV and 103 copies/ml of Dengue virus. Several tested pestiviruses, which also belong to the Flaviviridae family, and other RNA viruses such as the Chikungunya virus, which gives similar clinical symptoms as DENV, and the avian viruses APMV 2, 3 & 6, H5N1 & H7N1 and NDV gave no appreciable signal. To benchmark the present broad detection assay, flavivirus detection from the 65 clinical samples were compared with the detection obtained with dedicated TaqMan PCR assays (2-4). This showed 92% were detected with both methods. Only 68% of samples that were detected by the present method were detected by the benchmarking TaqMan systems (2-4) while three samples were negative by the former but detected by latter.

Conclusion: A simple broad detection method for flaviviruses was developed. It show excellent sensitivity as evaluated using the QCMD panels for WNV and Dengue virus and also high specificity using a broad range of RNA viruses including pestiviruses. The assay was successful with clinical materials varying from brain tissue to insect vectors. Compared to dedicated standard TaqMan based assays the sensitivity was superior. However, this is largely explained by one of the TaqMan system used (2) failed to detect a set of twelve samples from a recent WNV outbreak in Europe in 2008/2009. Otherwise it is likely that the presently described assay has a comparable sensitivity to TaqMan PCR and the outstanding virtue of this novel method is the broad detection of this highly variable virus genus.

References:
1-Yacoub. A. et al 2012. Arch Virol. 157: 833–844.
2-Chao. D.Y. et al 2007. J ClinMicrobiol 45(2) 584-589.
3-Brinkley C. et al 2008. IJMM 298: 73-80.
4-Unpublished

Demographics of natural oral infection of mosquitos by Venezuelan equine encephalitis virus

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RNA viral infections generally generate large and diverse populations within the infected host. This diversity plays a key role in important epidemiological and evolutionary processes. However, several steps during transmission can constrain the demographics and genetics of the virus population, with host primary infection being

one of the main barriers. During primary infection, the number of initially infected cells is not infinite, potentially lowering the size and genetic diversity of the colonizing population compared to that present in the donor host. The multiplicity of cellular infection (MOI) in those cells is thus a fundamental parameter determining the demographics and genetics of the colonizing population. The MOI is the number of genomes of a virus that enter and replicate in a cell. This parameter impacts the size of population bottlenecks during primary infection because, for a given number of primary infected cells, the higher the MOI, the larger the colonizing population. Furthermore, the MOI also influences genetic diversity as it largely defines the intensity of genetic exchange and complementation among genotypes during cell co-infection. Despite the importance of the MOI and population bottlenecks, there is a striking lack of formal estimates of these parameters, not only during primary infections but throughout the virus transmission cycle. Here, we use available datasets to estimate the demographics of Venezuelan equine encephalitisvirus (VEEV), a mosquito-borne arbovirus, during the primary oral infection (i.e. the midgut infection) of its mosquito vectors. We estimated the MOI during primary infection in the two transmission cycles using the method developed in Gutiérrez et al. (2010), and we use the results to model the potential for within-cell interactions among viral genotypes during primary infection. We also estimated the population bottleneck Ne endured by VEEV during bloodmeal ingestion and midgut infection. The methodology used is based on Fst statistics and uses genetic variance within and between populations (i.e. the virus populations in the inoculum and midguts) to estimate the effective population size. Despite of the fact that severe bottlenecks, in the order of single digits, are the rule during primary infection in the few virus models analyzed so far, VEEV population sizes were between one and two orders of magnitude higher. Larger populations at primary infection could be crucial during the arboviral cycle of VEEV because they can preserve diversity and facilitate adaptation during the compulsory alternation between arthropod and vertebrate hosts.

Simbu sero-group virus serum neutralizing antibodies in cattle in Tanzania

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Objective: Simbu sero-group viruses occur worldwide and include the newly recognized Schmallenberg virus (SBV) in Europe. These viruses cause in utero congenital malformations and reproductive losses in ruminants. Information on presence of these viruses in Africa is scarce and the origin of SBV is unknown. The aim of the present study was to investigate the presence of antibodies against SBV and closely related viruses in cattle in Tanzania, and their possible association with reproductive disorders.

Methods: Serum samples from 659 cattle in 202 herds collected in 2012/2013 were analyzed using a commercial SBV ELISA. The possible association between ELISA positivity and reproductive disorders were investigated by use of univariable logistic regression. Sera from 130 animals from the same area collected in 2008/2009, before the SBV epidemic in Europe, were also included and tested in the same ELISA. To interpret the ELISA results, SBV virus neutralization test (VNT) was performed on 110 sera from 2012/2013 and 71 from 2008/2009. In order to investigate the potential cross-reactivity with related viruses, 45 sera from 2012/2013 were analyzed in a series of VNTs, including viruses were Aino virus, Akabane virus, Douglas virus, Peaton virus, SBV and Tinaroo viruses.

Results: In the SBV ELISA test, 61% of the sera were positive. A significant association was found between ELISA seropositivity and reproductive disorders (OR= 1.9). Out of the samples from 2008/2009, 55% were positive. When analysed in SBV VNT, 51% of the samples from 2012/2013 and 21% of the samples from 2008/2009 were positive.

When analyzed in series of VNTs, antibodies against all six simbu sero-group viruses were detected. All sera