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Non-coding RNA determines flavivirus transmission by mosquitoes

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Purpose: Flaviviruses like West Nile, Zika, yellow fever and dengue virus produce an abundant non-coding subgenomic flavivirus RNA (sfRNA) in infected cells. SfRNA results from stalling of the host 5'-3' exoribonuclease XRN1/Pacman on conserved RNA structures in the 3'UTR of the viral genomic RNA. SfRNA production is conserved in insect-specific, mosquito- and tick-borne, as well as no-known-vector flaviviruses, suggesting a pivotal role for sfRNA in the flavivirus life cycle. Here we investigated the function of sfRNA during West Nile virus (WNV) infection of *Culex pipiens* mosquitoes and evaluated its role in determining virus transmission.

Methods & Materials: An sfRNA-deficient WNV was generated and compared to wildtype WNV for replication rates in cell lines. Infectivity and transmissibility was assessed in mosquitoes by measuring virus titers in the body and saliva.

Results: The sfRNA-deficient WNV displayed similar growth kinetics as wildtype WNV in both RNAi-competent and RNAi-compromised mosquito cell lines. Importantly, we demonstrate that the sfRNA-deficient virus displayed significantly decreased mosquito infection and transmission rates *in vivo* when administrated via the blood meal. Infection and transmission rates were not affected by sfRNA after intrathoracic injection.

Conclusion: This study identified sfRNA as a key driver to overcome the mosquito midgut infection barrier. This is the first report to describe a key biological function of viral, non-coding RNA in mosquitoes, providing an explanation for the strict conservation of sfRNA production in all flaviviruses.

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Novel laboratory methods to study/describe the molecular characterization of African swine fever virus isolates for the purposes of genotyping in Georgia

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Purpose: Since pig farming is inexpensive/efficient, pork and pork products are essential to the food supply of Georgia. Recently, swine in Georgia suffered from African swine fever (ASF), an exotic and especially dangerous communicable disease. Spread of this infection is a global concern and may reoccur in Georgia. The causative virus, ASF virus (ASFV), is a large, double-stranded, enveloped, icosahedral virus; it is the only DNA arbovirus. Molecular and genetic study (VP72-based) revealed 23 genotypes of the virus. According to the current data, outbreaks are usually caused by genotypes I and II. The outbreak in Georgia was caused by genotype II (Dixson *et al.*, 2008), which has a high potential for spread. There are several approved diagnostic methods used by the Laboratory of the Ministry of Agriculture (LMA) for testing for ASFV including an enzyme-linked immunosorbent assay and a virus specific polymerase chain reaction. The goal of the study was to implement new ASFV genotyping methods at LMA, thereby providing the opportunity to conduct laboratory based molecular/genetic study of ASFV for the first time in Georgia.

Methods & Materials: We selected twenty ASFV-containing samples for testing, which were collected in different regions of Georgia from 2007-2009. Three loci of the ASFV genome were used for the new genotyping method: 1) C-terminus of *B646L*, which encodes protein p72 (Bastos *et al.*, 2003); 2) *E183L* which encodes protein p54 (Gallardo *et al.*, 2009); and 3) central variable region primers.

Results: As expected, band sizes in the twenty ASFV positive samples were typical for genotype II viruses.

Conclusion: This research allowed us to conduct laboratory-based studies to identify molecular and genetic characteristics of ASFV in Georgia for the first time. If necessary, LMA will conduct similar studies in the future in Georgia**.** In case of recurrence of the disease, we will be able to determine the genotype of the circulating virus and be able to identify any changes in the virus genotype.

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The entomological surveillance as a tool for the early detection of mosquito-borne diseases: the experience of Piemonte, Liguria and Valle d'Aosta (Northwestern Italy)

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Purpose: This work describes the entomological surveillance carried out in Piedmont, Liguria and Valle d'Aosta (Northwestern Italy) that allowed the detection of West Nile Virus (WNV) before the occurrence of human cases. This diagnostic approach is focused to all major mosquito-borne flaviviruses of medical interest.

Methods & Materials: During the vector season, mosquito traps were located fortnightly in selected sites according to risk factors. Adult females were counted, identified to the species level and pooled by collection site, date and species with a maximum of 100 specimens. After RNA extraction, pools were analysed by Real Time RT-PCR distinctive for WNV Lineage 1 and Lineage 2 and USUTU

virus (USUV). Furthermore, a *Flavivirus* End-point RT-PCR was performed on pools collected in higher risk sites for the introduction of exotic invasive species and pathogens (international airports, ports and connection points, infectious diseases hospitals). Positive pools were sequenced to confirm the specificity of reaction and sent to the National Reference Centre for Animal Exotic Diseases (CESME, IZSAM) for confirmation.

Results: Since 2011, about 2,700 pools were analysed. The analysis revealed USUV circulation in Piedmont since 2011 and in Liguria since 2014. In 2014, WNV Lineage 2 was detected in Piedmont (Alessandria province) and Liguria (Genoa province). In 2015, the same virus was found in four different Provinces of Piedmont, showing an expansion of its activity and, at the end of the vector season, the first human case was confirmed. Following WNV detection in mosquitoes, as provided by national legislation, veterinary and human health control measures were activated (screening of equine sera and blood transfusion).

Conclusion: The surveillance network allowed to early detect the presence of mosquito-borne flaviviruses potentially pathogenic for humans and animals and provided useful information to public authorities, in order to apply control measures. With particular reference to WNV, our results showed that entomological surveillance can detect the virus in mosquitoes much earlier than in humans, as reported in other Italian regions. Moreover, considering the emergence caused by other flaviviruses of medical interest like Zika or Dengue, the ability of this surveillance protocol to detect *Flavivirus* spp. could represent an important tool for Public health authorities.

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Life cycle of Zika virus in human dendritic cells

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Purpose: Zika virus (ZIKV) is an emerging mosquito-borne *Flavivirus* causing birth defects and severe neurological complications in adults. Dendritic cells (DCs) act as major initiators and regulators of the immune response towards viral infections. There is at present very limited data on the interaction of ZIKV with DCs and possible immune evasion mechanisms. Here, we analyze the replication of ZIKV in DCs and their antiviral response with a particular focus on the comparison between the African and Asian strains of ZIKV.

Methods & Materials: Human monocyte-derived DCs were generated from the PBMCs of blood donors and infected with Asian (H/PF/2013) and African (M/Uganda/1962) ZIKV strains. Viral RNA load and interferon (IFN) type I and III responses were assessed by RT-PCR. Viral shedding was determined by a titration assay and markers of DCs activation/maturation and ZIKV-induced cell death were measured by a flow cytometry approach.

Results: We found that DCs were more susceptible to the Asian ZIKV strain in comparison to the African strain. Viral loads over time were similar for both strains. However, virus shedding by infected DCs was higher after infection with the African vs. Asian strain. Interestingly, infection with the Asian ZIKV strain induced lower IFN responses than the African strain. Neither activation of DCs nor cell death was induced after infection by both virus strains.

Conclusion: DCs are more susceptible to infection with the Asian ZIKV in comparison to African strain. In addition, the IFN responses after infection with the Asian strain of ZIKV are reduced in comparison to the African strain, which may explain the increased susceptibility of DCs to this strain. Although these results provide important information on how the ZIKV strain currently circulating in the Americas may evade the innate immune response, further investigation of ZIKV cellular targets are urgently required.

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