Detection of sequences of novel insect *Flaviviruses* from *Uranotaenia macfarlanei*, known as frog feeding mosquitoes, in Okinawa, Japan

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Statement of the Problem: Infectious diseases caused by mosquito-borne Flaviviruses, including *Dengue virus, Japanese encephalitis virus, Zika virus*, and Yellow fever virus, represent a worldwide public health threat. According to ecological changes and rapid increases in their incidence and geographic distribution, *Flaviviruses* are classified as emerging or re-emerging pathogens. The mechanisms underlying winter period maintenance and sylvatic transmission remain unclear. Okinawa is in the southern part of Japan, includes small islands, and has a subtropical climate, which allows some vector mosquitoes to survive year-round. Based on the vulnerability of isles against external stimuli including the invasion of known and unknown pathogens, we initiated a project for the comprehensive and highly sensitive detection of pathogens from field-caught mosquitoes.

Methodology & Theoretical Orientation: In 2015 and 2016, we collected 3396 mosquitoes from 33 sentinels in Okinawa Island. These mosquitoes were morphologically and genetically identified and pools were made (max 20). RNA was extracted from each pool, RT-BT-PCR was conducted with *Flavivirus* universal primers targeting the NS5 protein gene (Kuno), and positive PCR products were sequenced.

Findings: We detected 4 nucleotide sequences of novel *Flaviviruses* from *Uranotaenia macfarlanei* spp, known as frog-feeding mosquitoes, near a cave in Okinawa Island in June, Sept, and Oct. 2016. A BLAST search and phylogenetic analysis of the NS5 protein using the Neighbor Joining method, showed that the sequences created Okinawan-specific clusters within the clade of insect-specific *Flaviviruses*, and showed the highest homology with Nakiwogo virus isolated from Mansonia Africana, Uganda. Attempts to isolate the virus were unsuccessful.

Conclusion & Significance: Four sequences were detected at different times in one location; therefore, viral circulation was established in the area, possibly between frogs and *Uranotaenia*. These are distinct from other pathogenic agents of mammals including humans. Pokilotherms may play important roles in maintaining sylvatic forms of *Flaviviruses*.

Speaker Biography

Mika Saito is a Veterinarian and Assistant Professor in the Department of Virology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan. She has graduated and received a PhD from the Graduate School of Veterinary Medicine, Hokkaido University in 1986 and 2009, respectively, and received an MA in International Development from Nihon Fukushi University, Aichi, Japan in 2002. Her major research interest includes risk assessments of infectious diseases caused by mosquito-borne *Flaviviruses* such as Japanese *encephalitis* virus and virus, including human and environmental factors, such as socio-economics and human lifestyles, and relationships with wild life. She is a Group Leader of mosquito research group in the project "development for the control strategy of vector-borne and zoonotic diseases in Okinawa and formulation of networking among stakeholders Okinawa Communicable Disease Research Hub Formation Promotion Project since 2015". She is attempting to combine IT and AI for risk assessments and the control of diseases.

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