# Optimizing Flavivirus Detection in Free-ranging Non-Human Primates

Olivia Cords[1], JoAnn Yee[2], Peter Nham[2], Pranav Pandit[3], Tierra Smiley Evans[3]

UC Davis School of Veterinary Medicine[1], Primate Assay Laboratory Core, California National Primate Research Center[2], One Health Institute, University of California, Davis, California, United States of America[3]

## Introduction:

Flavivirus transmission can include a wide variety of species, with sylvatic cycles often complicating prevention and control efforts in humans[1,2]. This research examines the traits of non-human primates (NHP) with confirmed detection of flaviviruses and uses non-invasively collected oral samples to optimize a serologic assay to help target and improve future surveillance efforts.

## Objectives:

- Aim 1: Optimize a non-invasive saliva method to detect exposure to flaviviruses in NHP.
- Aim 2: Curate a comprehensive dataset of NHP species, their host traits, and evidence of detection of flaviviruses.
- Aim 3: Analyze ecological and biological traits associated with detection of flaviviruses in NHP species.

## Methods:

**Optimizing non-invasive saliva method:**

- Saliva samples were collected from macaques in Southeast Asia using a non-invasive rope method[3], as well as conventional serum and oral samples.
- Samples were tested for antibodies with 5 different magnetic bead-based Luminex assays.
- Plaque reduction neutralization assays (PRNT) were performed to confirm suspect CHIKV positives.

**Ecological Traits Analaysis:**

- A dataset was created using the "Ecological Traits of the World's Primates" dataset[4], the IUCN Red List of Species[5], and a literature review of flaviviruses detected in NHP.
- A multivariable zero-inflated Poisson model was used to assess relationship between NHP traits and flavivirus richness (sum of flaviviruses in each species).

## Results:

**Agreement between oral swabs (tested with NHP IgGMA) versus serum (tested with human IgG)**

| Antigen | Kappa value | Strength of agreement [7,8] |
|---|---|---|
| Zika NS1 (Antigen 1) | -0.01 | poor |
| DENV1 NS1 | -Inf | |
| Zika NS1 (Antigen 2) | 0.32 | fair |
| Zika env (b) | 0.27 | fair |
| DENV2 NS1 | -0.01 | poor |
| DENV3 NS1 | -0.01 | poor |
| WNV NS1 | -0.01 | poor |
| JEV NS1 | 0.80 | substantial |
| YFV NS1 | -0.02 | poor |
| TBEV NS1 | -0.03 | poor |
| CHIKV-E1 (Antigen 1) | 0.39 | fair |
| CHIKV-E1 (Antigen 2) | 0.85 | almost perfect |
| DENV4 NS1 | 0.66 | substantial |

- CHIKV antibodies were confirmed with PRNT in 5 non-invasive saliva samples and 9 serum samples.

**Zero-inflated Poisson model analyzing ecological traits and flavivirus richness***

| Traits | IRR | 95% CI | P value |
|---|---|---|---|
| (Intercept) | 0.27 | (0.10, 0.75) | 0.011* |
| log(Pubmed hits) | 1.39 | (1.20, 1.60) | <0.001*** |
| subtropical/tropical heavily degraded former forest | 2.87 | (1.67, 4.75) | 0.008** |
| Savanna | 2.32 | (1.21, 4.47) | 0.012** |
| subtropical/tropical dry lowland grassland | 6.22 | (1.95, 1.98) | 0.002** |
| log(Mean individuals/group) | 0.54 | (0.34, 0.85) | 0.008** |

*Data analyzed included 526 species (10% of which were positive for flaviviruses), 11 ecological traits, and 40 habitats.

## Conclusions and Future Aims:

- Antibodies against CHIKV were detected from non-invasively collected saliva samples and validated with PRNT for the first time.
- Subtropical/tropical heavily degraded former forests, savanna, and subtropical/tropical dry lowland grassland habitats are positively associated with flavivirus richness. Mean individuals per group are negatively associated.
- In the future, conduct PRNT assays for other flaviviruses.

## References:

[1] Samir, et al. "The global distribution and burden of dengue." Nature 496.7446 (2013): 504-507.

[2] Weaver, S. C. & Barrett, A. D. T. Transmission cycles, host range, evolution and emergence of arboviral disease. Nat. Rev. Microbiol. 2, 789–801 (2004).

[3] Pandit, Pranav S., et al. "Predicting wildlife reservoirs and global vulnerability to zoonotic Flaviviruses." Nature communications 9.1 (2018): 1-1

[4] Smiley Evans, Tierra, et al. "Optimization of a novel non-invasive oral sampling technique for zoonotic pathogen surveillance in nonhuman primates." PLoS neglected tropical diseases 9.6 (2015): e0003813.

[5] Galán-Acedo, C., Arroyo-Rodríguez, V., Andresen, E. et al. Ecological traits of the world's primates. Sci Data 6, 55 (2019). https://doi.org/10.1038/s41597-019-0059-9

[6] International Union for the Conservation of Nature (IUCN). The IUCN Red List of threatened species. Version 2020. See http://ww.iucnredlist.org

[7] Altman, Douglas G. 1999. *Practical Statistics for Medical Research*. Chapman; Hall/CRC Press.

[8] Landis JR, Koch GG. 1977. "The Measurement of Observer Agreement for Categorical Data" 1 (33). Biometrics: 159–74.

## Acknowledgements:

Research Grant: NIH T32 Medical Scientist Training Program Grant 2020-2021 (NIH Grant T32GM136559)

Student Support: UC Davis School of Veterinary Medicine, Students Training in Advanced Research Grant