

## Background

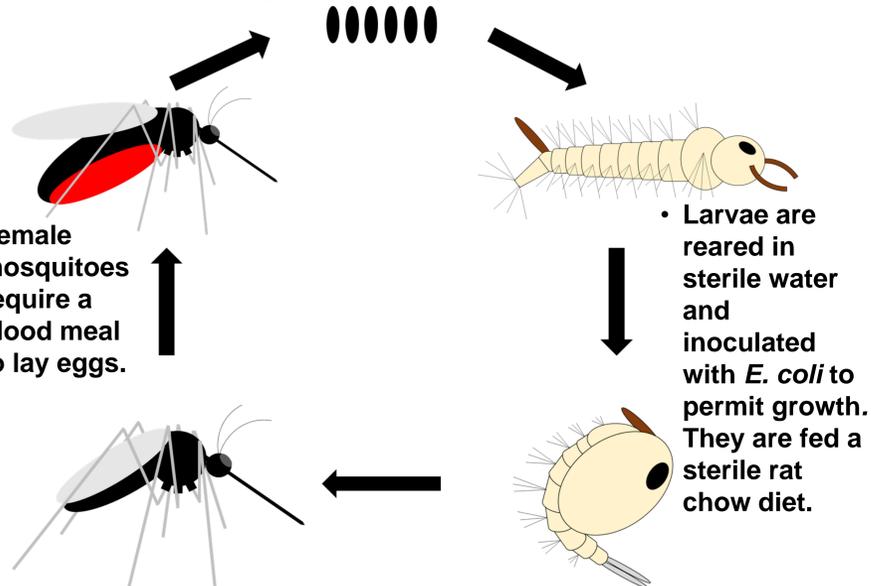
The mosquito *Aedes aegypti* is the primary vector of dengue virus (DENV), which causes disease in humans. Prior studies report increased susceptibility of *A. aegypti* to DENV infection<sup>1</sup>, and higher pathogen burden<sup>2</sup> when adult females are treated with antibiotics. This suggests a role for the microorganisms that colonize the digestive tract of mosquitoes (gut microbiota) in susceptibility to DENV infection. However, no studies have examined whether axenic mosquitoes with no gut microbiota are more susceptible to DENV.

## Objectives

We tested the hypothesis that axenic of *A. aegypti* are more susceptible to DENV infection by comparing three treatments: axenic mosquitoes with no gut microbiota, gnotobiotic mosquitoes containing only *Escherichia coli*, and conventional mosquitoes with a natural community of gut microbes. We validated the status of these mosquitoes using both culturing methods and PCR. We then assessed virus presence/absence of DENV in each treatment after feeding adult females an infected blood meal. If our hypothesis is correct, axenic mosquitoes should be more susceptible to infection than gnotobiotic and conventional mosquitoes.

## Sterile Mosquito Rearing

- Mosquito eggs are washed with multiple washes of sterile water, concentrated bleach, the veterinary disinfectant Roccal-D, and 70% ETOH.



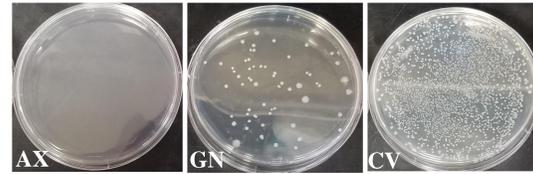
Female mosquitoes require a blood meal to lay eggs.

Larvae are reared in sterile water and inoculated with *E. coli* to permit growth. They are fed a sterile rat chow diet.

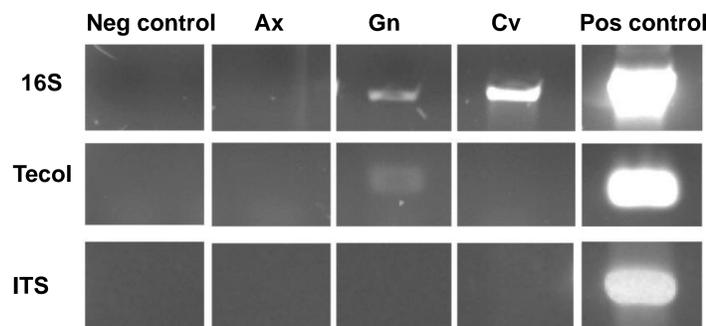
Adult mosquitoes are kept in a sterile environment and fed a sterile 5% sucrose solution.

Pupae are surface-sterilized with 2% bleach. They are then treated with ampicillin and transferred to a sterile adult cage to emerge.

## Validation of microbial status

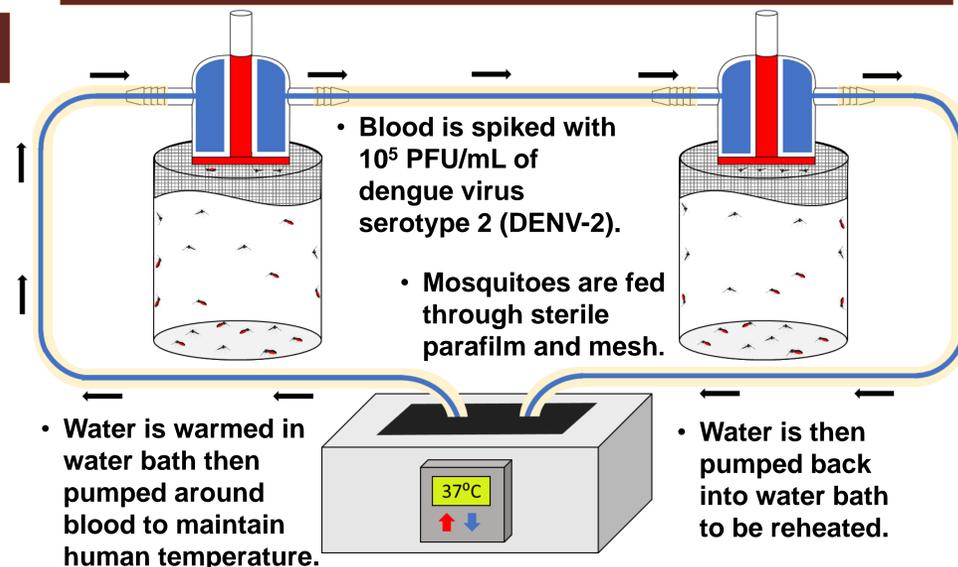


- Test for microbial growth on LB-agar petri dishes. AX: Axenic. GN: Gnotobiotic. CV: Conventional



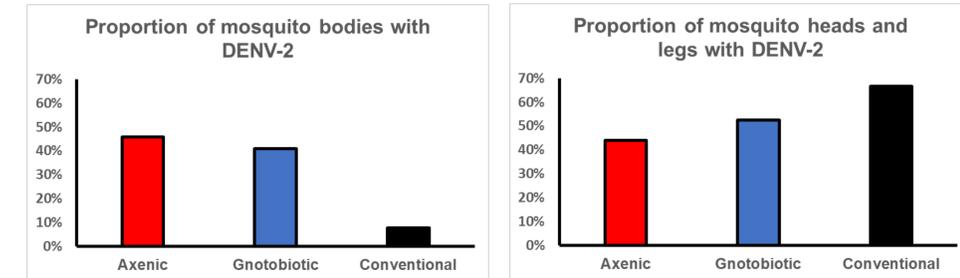
- PCR to detect microbial DNA. 16S: universal bacterial primers. Tecol: *E. coli*-specific primers. ITS: universal fungal primers.
- Negative control for PCR: water. Positive control for PCR: *Escherichia coli* culture extract (16S and Tecol primer sets), *Saccharomyces cerevisiae* culture extract (ITS primers).

## Mosquito infection with DENV



- Dengue presence test is shown above. Mosquito bodies are homogenized two weeks after ingesting infected blood. RNA is extracted from the homogenate and converted to cDNA. This is then tested with specific dengue primers. Positive samples show a band at 511 bp.

## Results



- $p < 0.0001$  (Fisher's Exact Test)
- Indicates viral uptake into mosquito heads and legs.
- $p = 0.74$  (Fisher's Exact Test)
- Indicates viral dissemination into heads and legs.

## Conclusions

Consistent with our working hypothesis, a larger proportion of axenic mosquitoes were infected with DENV-2 than conventional mosquitoes. In contrast, the proportions of axenic and gnotobiotic mosquitoes infected with DENV were nearly identical. These results suggest *A. aegypti* with a more diverse gut microbiota are more resistant to DENV infection than axenic females but *E. coli* alone provides no increase in resilience. However, our treatments had no effect on DENV dissemination among females that were infected. Further experiments are required to expand on these results. First, RT-PCR does not provide an estimate of the number of infectious DENV particles that were produced in each treatment. Thus, cell-culture based assays also need to be conducted. Another appropriate step would be to test mosquito saliva as this alone determines the ability of a mosquito to transmit DENV.

## Acknowledgements

Thanks to Dr. Jamie Snyder, Dr. Nancy Buckley, and Dr. Jill Adler for their guidance and assistance. Thanks MBRS RISE program for providing me the opportunity to apply to the NSF REU program. This research was funded by the NSF.

## References

- Dong, Yuemei, et al. "Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites." *PLoS Pathogens*, vol. 5, no. 5, Aug. 2009, doi:10.1371/journal.ppat.1000423
- Gendrin, Mathilde, et al. "Antibiotics in Ingested Human Blood Affect the Mosquito Microbiota and Capacity to Transmit Malaria." *Nature Communications*, vol. 6, no. 1, June 2015, doi:10.1038/ncomms6921

## Contact Information

rdmanuel@cpp.edu