Influence of mosquito gut microbiota on susceptibility to dengue infection
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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 infection¹, and higher pathogen burden² 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
- 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
- Blood is spiked with 10⁶ PFU/mL of dengue virus serotype 2 (DENV-2).
- Mosquitoes are fed through sterile parafilm and mesh.
- Water is warmed in water bath then pumped around blood to maintain human temperature.
- Water is then pumped back into water bath to be reheated.
- 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
- Proportion of mosquito bodies with DENV-2
- Proportion of mosquito heads and legs with DENV-2
- p < 0.0001 (Fisher’s Exact Test) Indicates viral uptake into mosquito midgut.
- 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.

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References

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