INTRODUCTION

- Yellow fever virus (YFV) is predominantly transmitted to humans by Aedes species mosquitoes.
- Climate change projections suggest that the habitat and transmission range of Ae. aegypti will expand, leading to higher risk and burden of the viruses they transmit, like YFV.
- To prevent outbreaks in endemic regions, the live attenuated vaccine, YFV-17D is used because it is highly efficacious and safe.
- Previous work showed that YFV-17D replicates in the midgut of Ae. aegypti but disseminates poorly out of the midgut when compared to wild-type YFV strains.
- It has been shown for other arboviruses, that increased temperature can increase infection, dissemination, and transmission rates, and levels of virus in mosquito samples.
- The degree to which increased temperature may alter the replication and dissemination of YFV-17D in Ae. aegypti has not been identified.

We aim to determine the impact of increased temperature on YFV-17D replication and dissemination rates in Ae. aegypti.

METHODS

Mosquito Experimental Overview

Mosquitoes were moved into BSL3 environment pre-eclosion, allowing adjustment to different temperature before infection with YFV-17D via blood meal. Midgut, legs, and saliva were collected on days 7 or 14 post-infection depending on the experiment. RNA extractions were performed and viral RNA detected and quantified using qRT-PCR with YFV-specific primers and probe.

RESULTS

Increased YFV-17D replication in midgut and significantly higher levels of disseminated virus at higher temperature

Figure 3: On days 7 and 14 post-infection, A/B) midguts and C/D) legs & wings were collected, RNA extracted, and YFV-17D vRNA was quantified with qRT-PCR (day 7, n=24; day 14, n=14). Samples with detectable YFV-17D are plotted (mean ± SD shown in black). Dotted line indicates the limit of detection. Unpaired t-tests were performed between temperatures. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.0001).

Higher average of YFV-17D levels in midgut at moderately higher temperature

Figure 5: On day 14 post-infection, A) midguts, B) legs & wings and C) saliva were collected, RNA extracted, and YFV-17D vRNA was quantified with qRT-PCR (n=36). Samples with detectable YFV-17D are plotted between temperatures (excluding saliva due to low positive sample numbers). Unpaired t-tests were performed between temperatures.

CONCLUSION

- When compared to Ae. aegypti kept at 29°C, mosquitoes kept at 34°C had higher rates of YFV-17D dissemination from the midgut.
- Additionally, at 34°C, YFV-17D replicated to significantly higher levels in the midgut, and the legs & wings compared with mosquitoes kept at 29°C.
- In Ae. aegypti kept at 32°C, YFV-17D has higher rates of infected dissemination when compared to mosquitoes kept at 29°C.

At higher temperatures YFV-17D has higher rates of dissemination, indicating that the vaccine is able to overcome the midgut escape barrier.

Future studies will focus on determining if higher temperatures allow for viral replication in the salivary glands and dissemination into saliva. Additional infectivity assays will be performed, to ascertain if the viral RNA detected in these experiments correlates to infectious virus. Moreover, YFV-17D has many attenuating mutations compared to virulent strains. We’re interested in exploring if these mutations are maintained in the populations at each barrier to see if increased dissemination is related to reversion of attenuating mutations.

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