



June 2, 2020

Journal of Visualized Experiments

Dear Editor,

We are happy to submit the revised version of our manuscript entitled "Propagation of the microsporidian parasite *Edhazardia aedis* in *Aedes aegypti* mosquitoes" for publication in JoVE as a part of the Methods Collection "The Study of Mosquito Biology."

In this work, we detail the method for laboratory culture of the microsporidian parasite *Edhazardia aedis*. We demonstrate successful propagation of infection by quantifying *E. aedis* prevalence and parasite load in *A. aegypti* mosquitoes. *E. aedis* is an obligate parasite of *Aedes aegypti* and the parasite must be reared in live mosquitoes. Rearing of the parasite in mosquitoes requires controlled dosing of mosquitoes each generation and careful quantification of parasite loads. The general method has been published elsewhere, however, we feel that previous attempts to describe the rearing method have not been as detailed as would be necessary for a novice experimenter to be successful without substantial trial and error. We believe that the attached protocol will offer valuable clarification and detail to assist both novice and experienced researchers alike.

It is our hope that publishing this detailed protocol with high quality video and visual aids will increase accessibility to the practice of rearing *E. aedis* and allow more researchers to investigate the basic biology and applied potential of this system.

We have considered and addressed all suggestions from yourself and the reviewers, and we believe the manuscript is improved from its original version.

We appreciate your consideration of our revised work.

Best wishes,

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