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Title: Propagation of the Microsporidian Parasite Edhazardia aedis in Aedes aegypti Mosquitoes

Authors and Affiliations:

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Leica DM300

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? **Next door to each other**

Current Protocol Length

Number of Steps: 9
Number of Shots: 25

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Sarah Short:** *Edhazardia aedis* is a microsporidian parasite that specifically infects *Aedes aegypti* mosquitoes. Rearing this parasite can be challenging for new researchers, due to the complexity of the parasite's life cycle.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Brendan Kelly:** This protocol provides stepwise instructions for rearing *E. aedis*, including how to properly time all steps of the procedure, identify infectious spores, and propagate the next generation of parasites.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Brendan Kelly:** Accurate timing of this protocol can be challenging and may require some trial and error. Identifying spores can also be difficult, but visual aids in this protocol should provide assistance.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Days 0 – 1: Hatch *Ae. aegypti* eggs infected with *E. aedis*

2.1. To begin, hatch *Ae. aegypti* eggs infected with *E. aedis* by placing them in a larval rearing tray with 1 liter of deionized water **[1]** and adding 50 milligrams of fish food **[2]**. House the mosquitoes at 27 degrees Celsius and 80% relative humidity with a 14-hour light-10-hour dark cycle **[3]**.

2.1.1. Talent transferring eggs to a rearing tray.

2.1.2. Talent adding fish food to the rearing tray.

2.1.3. Talent putting the tray in the incubator and closing the door.

2.2. After hatching, reduce the density of larvae to approximately 100 larvae per tray, making new trays as necessary **[1]**. Add a piece of dry cat food to each tray. Replenish the food when depleted, but do not provide an excess of food. One 200-milligram piece of cat food every three days is sufficient **[2]**.

2.2.1. Talent making new trays.

2.2.2. Talent adding cat food to trays.

3. Days 4-5: Hatch uninfected *Ae. aegypti*

3.1. When the infected larvae are 3rd to 4th instars, hatch uninfected *Ae. aegypti* eggs in a new tray **[1]**. Rear the mosquitoes at densities such that healthy *Ae. aegypti* reach 2nd to 3rd instar in 48 to 72 hours. Hatching batches of healthy eggs over multiple days can guarantee that larvae are at the correct stage when needed **[2]**.

3.1.1. Talent putting uninfected eggs in a tray.

3.1.2. Mosquitoes of multiple ages in 2-3 trays.

4. Days 7–8: Horizontal Transmission

4.1. To harvest and quantify uninucleate spores, use a transfer pipette to move 10 infected 4th instar larvae to a 1.5-milliliter microcentrifuge tube **[1]**. Remove the breeding water with a transfer pipet **[2]** and wash the larvae once by adding approximately 1 milliliter of clean deionized water **[3]**.

4.1.1. Talent transferring larvae to a tube.

4.1.2. Talent removing the breeding water.

4.1.3. Talent washing the larvae.

4.2. Remove the wash water **[1]**, add 500 microliters of clean deionized water to the 10 larvae **[2]**, and homogenize them using a pestle and a mechanical homogenizer **[3]**. Load 10 microliters of homogenate onto a hemocytometer **[4]** and quantify the spores at 400 X magnification **[5]**. *Videographer: This step is important!*

4.2.1. Talent removing the wash water.

4.2.2. Talent adding clean water.

4.2.3. Talent homogenizing the larvae.

4.2.4. Talent pipetting homogenate onto hemocytometer

4.2.5. SCOPE: Spores that are being quantified. NOTE: Author uploaded video and images for this: 61574_4.2.5.

4.3. Make a fresh larval food slurry by mixing 1.2 grams of liver powder, 0.8 grams of brewer's yeast, and 100 milliliters of water **[1]**.

4.3.1. Talent making a larval food slurry.

4.4. Transfer 100 2nd to 3rd instar healthy *Ae. aegypti* larvae into 150 milliliter beakers or specimen cups **[1]** and add 5 x 10^{4} to 1 x 10^{5} spores to each cup with a pipet **[2]**. Add 2 milliliters of larval food slurry and deionized water for a final volume of 100 milliliters **[3]**. *Videographer: This step is important!*

4.4.1. Talent transferring larvae to a beaker.

4.4.2. Talent dosing the larvae with spores.

4.4.3. Talent adding food slurry and water to the larvae.

4.5. After 12 to 24 hours of exposure, transfer the larvae into rearing trays, add food, and rear them to adulthood **[1]**. Monitor the dosed larvae for pupation and transfer pupae to an emergence cup in a cage **[2]**. Sugar feed adults *ad libitum* **[3]**.

4.5.1. Talent transferring larvae to a rearing tray and adding food.

4.5.2. Talent transferring pupae to an emergence cup.

4.5.3. Talent administering food to mosquitoes.

4.6. Blood feed adults according to standard protocols available through BEI resources **[1]**. Collect eggs from blood fed adults. Vertical transmission of *E. aedis* occurs at this step **[2]**. When finished, clean all materials that came in contact with *E. aedis* with 10% bleach and autoclaving to prevent contamination **[3]**.

4.6.1. Talent placing membrane blood feeder on top of cage.

4.6.2. Talent removing eggs from cage and placing in bag for storage.

4.6.3. Talent cleaning materials with 10% bleach.

Results

5. Results: Effective *E. aedis* Infection in Filial Generation

5.1. This protocol demonstrates the full procedure necessary to propagate *E. aedis* in *Ae. aegypti* mosquitoes **[1]**. *Ae. aegypti* eggs infected with *E. aedis* were hatched, and larvae were reared to the 4th instar stage **[2]**. In the 4th instar stage, visual signs of infection could be observed such as white spore cysts throughout the fat bodies of infected larvae **[3]**.

5.1.1. LAB MEDIA: Figure 1.

5.1.2. LAB MEDIA: Figure 2 B.

5.1.3. LAB MEDIA: Figure 2 B. *Video Editor: Emphasize the infected larvae.*

5.2. Uninucleate spores were harvested from 4th instar larvae by homogenizing 10 larvae in 500 microliters of deionized water **[1]**. These spores were pyriform and readily visible at 400x magnification **[2]**. A spore count of 4050 spores per microliter was calculated and these spores were used to infect healthy larvae via horizontal transmission **[3]**.

5.2.1. LAB MEDIA: Figure 2 A.

5.2.2. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the spores that the red arrows are pointing at.*

5.2.3. LAB MEDIA: Figure 2 A.

5.3. Infection status and *E. aedis* loads of 25 filial generation larvae were assessed at 7 days post hatching **[1]**. Vertical infection rate of *E. aedis* in the filial generation was found to be 96% **[2]** and the mean spore load of infected individuals at seven days post hatching was 3.31 x 10^5 **[3]**.

5.3.1. LAB MEDIA: Figure 3.

5.3.2. LAB MEDIA: Figure 3 A.

5.3.3. LAB MEDIA: Figure 3 B.

Conclusion

6. Conclusion Interview Statements

6.1. **Sarah Short:** To properly coordinate larval staging, it is advisable to account for some variability in rearing time. Additionally, spore numbers increase rapidly, so if you don't detect spores, try again the next day.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.5.*