**Testing for evidence of a dilution effect with *Pasteuria ramosa***

Does the presence of *D. pulicaria*, *D. retrocurva*, and/or non-competent *D. dentifera* affect the prevalence of infection in competent *D. dentifera*?

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pulicaria  (Mid-D-61) | | | | |
| 0 | 2 | 4 | 6 | 8 |
| 0A | - | - | - | - |
| 0B | - | - | - | - |
| 0C | - | - | - | - |
| 0D | - | - | - | - |
| 0E | - | - | - | - |
| 0F | - | - | - | - |
| 0G | - | - | - | - |
| 0H | - | - | - | - |

8 replicates

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Retrocurva  (Field collected) | | | | |
| 0 | 2 | 4 | 6 | 8 |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| NC Dentifera  Gd107 | | | | |
| 0 | 2 | 4 | 6 | 8 |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |
| - | - | - | - | - |

1. Field collected Retrocurva (from North Lake) were placed into flasks with filtered water prior to the start of the experiment.
   1. These were collected on 9-9-15 and transferred to 100mL beakers on 9-9-15 as well.
2. Adults of each of the initial hosts are added to 100mL of filtered lake water. The number of hosts added to each beaker varies across a gradient (0, 2, 4, 6, and 8 hosts per beaker) with 8 replicates of each beaker set-up.
   1. Need to spread evenly across groups (in terms of the size of host)
   2. Set up an extra beaker in the 6 individual group
      1. Pick one of the beakers randomly from the 6 individual group and take pictures.
      2. Pictures should measure from center of eye to base of the tail spine.
   3. Beakers are kept in an incubator at 20°C on a16/8 light/dark cycle.
3. After all the hosts are added, 100,000 Pasteuria spores (isolate 18) are added to each beaker. 1 mL of algae (Ankistrodesmus) is added as well.
4. The exposure period for the initial hosts is 48 hours. (Starting 9-10-15 at 1:46pm and ending 9-12-15)
   1. They will be checked after 24 hours to remove any babies. This will be done with the smaller/tapered pipette to reduce the potential for removing parasite spores as well.
5. Following the 48 hour exposure period, initial hosts will be removed and placed in new beakers with clean water.
   1. They will be checked twice a week to remove babies and water will be changed once a week.
   2. Infected animals will be recorded and removed from the beakers.
6. Using the beakers that previously housed the initial hosts, we will add 5, neonate (<24 hours old) dentifera on 9-12-15. The exposure period will last 5 days, ending on 9-17-15.
7. After this exposure period, dentifera will be moved to new beakers with clean water. Similarly…
   1. They will be checked twice a week to remove babies and water will be changed once a week.
   2. Infected animals will be recorded and removed from the beakers.

Recent Addition

I’m going to keep several beakers retrocurva (exact number to be determined) as a backup. Some will be used to replace retrocurva that die during the experiment, but others will serve to gauge the amount of inapparent infections that we let through. i.e. individuals that were infected in the wild but recently enough to go undetected before we placed them in the experiment. This way we’ll have a comparison group in case a lot of retrocurva that are supposed to be “diluter hosts” end up getting infected.

Numbers to know

* 240 total beakers to keep track of
* 600 total dentifera needed
* 160 total animals for **each** other species (480 combined)
* 120 beakers to add spores to
  + 1000 spores/mL
  + 100,000 spores per beaker
* 12 million Pasteuria spores

Further notes

* If we are short on hosts, the 6 animal beaker treatments can be cut.
* Pulicaria in Friendly Competition clones (Mid D-61,64,67)

SCHEDULE

**Wednesday 9-9-15**

* Set up Retrocurva from North (collected today)
  + Do not refrigerate the gallon jugs
* Filter water
* Get incubator 4 up and running

**Thursday 9-10-15**

* Diluter hosts go in beakers
* Add Pasteuria spores

**Friday 9-11-15**

* Clear out babies from the Mid37 “Dilution Moms” in the incubator
  + Their new offspring will be added to the beakers on Saturday

**Saturday 9-12-15**

* Transfer diluter hosts to clean water beakers
  + Keep each beaker as a group
  + Remove babies if needed
* Add neonate offspring (Mid37 incubator) to the spore beakers

**Thursday 9-17-15**

* Remove Mid37 clones from exposure beakers and place in clean water.