**Final Project Paper**

*Introduction*

The purpose of this project is to determine if season and larval age have any effect on the livelihood of the parasite. Measuring the viability, exsheathment and hatchability of the worms and their eggs does this. Viability is calculated by watching the movement of worms after they were grown either in vivo or in vitro. Hatchability is calculated by seeing how many eggs hatch from feces collected from each animal on trial. Exsheathment is calculated by counting the number of worms that lose their sheath, or outer covering, after being exposed to carbon dioxide. Measuring these three parameters, both in vivo and in vitro, allows for an accurate representation of how the parasites are surviving during each season. The project was designed to run in a series of four cycles corresponding to the beginning of the four seasons of the year 1) Fall (autumnal equinox, September 22, 2017); 2) Winter (Winter solstice, December 21, 2017); 3) Spring (vernal equinox, March 20, 2018); 4) Summer (summer solstice, June 21, 2018). A fifth cycle was added as a replicate of the original fall cycle, which is termed ‘Fall 2’ (autumnal equinox, September 22, 2018).

*Methods*

Each cycle began (time (t) = 0) with the experimental infection of two donor lambs with 10,000 H. contortus L3 larvae obtained from previously infected donor animals. The larvae obtained from the previously donors was between 1 and 3 weeks. Fecal samples were collected from each lamb for both egg recovery and larval culture beginning at four weeks of infection and continuing every four weeks through 16-20 weeks of infection. Upon the collection of the fecal sample, eggs were extracted from the feces and used in the egg hatch assay to determine egg hatchability. The remainders of the fecal samples were used to prepare fecal cultures that yielded L3 larvae. The larvae were extracted from the culture samples and used in an *in vitro* exsheathment assay using CO2 treatment, as well as in an *in vivo* exsheathment assay using 4 ruminally fistulated ewes.

For the analysis of all the data collected, the following steps were taken. First the data was uploaded and split into smaller data sets based on the cycle the data was from and values that were not being shown. From there, a series of different models were created for each cycle. Within each cycle, Larval Age was compared against each of the independent variables; to see what effects would be shown. Models were then created to show the effect of both Larval Age and Cycle on each independent variable as well as combinations of them. Models were graphed and tables of significant values were created in the markdown.

*Table 1: List of models, their variables, and the datasets used for each one*

|  |  |  |  |
| --- | --- | --- | --- |
| Model | X Value | Y Value | Data Set |
| A | LarvaeAge | In Vitro Viability | cycle\_#NA\_vitro |
| B | LarvaeAge | In Vitro Exsheathment | cycle\_#NA\_vitro |
| C | LarvaeAge | In Vivo Viability | cycle\_# |
| D | LarvaeAge | In Vivo Exsheathment | cycle\_# |
| E | LarvaeAge | Hatchability | cycle\_#NA\_hatch |
| F | LarvaeAge | In Vitro Viability + In Vitro Exsheathment | cycle\_#NA\_vitro |
| G | LarvaeAge | In Vivo Viability + In Vivo Exsheathment | cycle\_# |
| H | LarvaeAge | In Vitro Viability + In Vivo Viability | cycle\_#NA\_vitro |
| I | LarvaeAge | In Vitro Exsheathment + In Vivo Exsheathment | cycle\_#NA\_vitro |
| J | LarvaeAge | In Vitro Exsheathment + In Vivo Exsheathment + In Vitro Viability + In Vivo Viability + Hatchability | cycle\_#NA\_hatch |
| K | Cycle | In Vitro Exsheathment + In Vivo Exsheathment + In Vitro Viability + In Vivo Viability | no\_cycle1 |
| L | LarvaeAge + Cycle | In Vitro Exsheathment + In Vivo Exsheathment + In Vitro Viability + In Vivo Viability | no\_cycle1 |
| M | LarvaeAge + Cycle | In Vitro Viability + In Vivo Viability | no\_cycle1 |
| N | LarvaeAge + Cycle | In Vitro Exsheathment + In Vivo Exsheathment | no\_cycle1 |
| O | LarvaeAge + Cycle | Hatchability | no\_cycle1 |
| P | LarvaeAge + Cycle | In Vitro Exsheathment + In Vivo Exsheathment + In Vitro Viability + In Vivo Viability + Hatchability | no\_cycle1 |
| Q | LarvaeAge + Cycle | InVivoExsheathment | no\_cycle1 |

*Results*

In the cycle specific models, there were mixed results based on the variables tested. In Cycle1, the only model that was statistically significant was model C1, Laval Age vs. In Vivo Viability with a pvalue of 0.02382. In Cycle2 significant models included A2, C2, F2, and H2 all with pvalues of less than 0.05. Cycle3’s significant models include A3, B3, C3, F3, G3, H3, I3, J3 again all with pvalues of less than 0.05. Models with pvlaues below 0.05 for Cycle4 were B4, F4, I4 and J4. In Cycle 5 significant models include B5, F5, and J5. In the intercycle models, we determined that models K, L, and M were all statistically significant with pvalues of less than 0.05.

*Table 2: Models that showed Statistical Significance and their values*

|  |  |  |
| --- | --- | --- |
| Model | P-value | R-squared |
| C1 | 0.02382 | 0.1881 |
| A2 | 0.001279 | 0.3449 |
| C2 | 0.02108 | 0.1468 |
| F2 | 0.004005 | 0.3687 |
| H2 | 0.00251 | 0.3928 |
| A3 | 0.01979 | 0.1791 |
| B3 | 0.001079 | 0.3218 |
| C3 | 2.04\*10^-5 | 0.3835 |
| F3 | 0.0003439 | 0.4461 |
| G3 | 9.547\*10^-5 | 0.3937 |
| H3 | 0.0006569 | 0.4189 |
| I3 | 0.005206 | 0.3226 |
| J3 | 0.0445 | 0.7942 |
| B4 | 8.23\*10^-11 | 0.7837 |
| F4 | 1.038\*10^-9 | 0.784 |
| I4 | 1.083\*10^-10 | 0.8173 |
| J4 | 0.04933 | 0.8874 |
| B5 | 1.96\*10^-5 | 0.4496 |
| F5 | 0.0001286 | 0.4497 |
| J5 | 0.02211 | 0.9262 |
| K | 0.05036 | 0.3159 |
| L | 0.008215 | 0.4234 |
| M | 0.002364 | 0.372 |

*Discussion*

It was determined previously that Cycle 1 wasn’t producing accurate data, which is why the Fall measurements were replicated in Cycle 5. This is reflected in the fact that only one model had any level of significance, Laval Age tested against In Vivo Viability. If we only pay attention to the results of Cycles 2-5 we will get a more accurate representation of what the actual effects of seasonality are. Across all cycles, there was a shown significant effect of cycle on In Vitro Viability and In Vitro Exsheathment. Because in vitro experiments were more strongly controlled and less variable than the in vivo experiments, it is likely that this stability might have had a small effect on the results. Cycle 3 had the most models with significant values, with all models except D and E having p-values of less than 0.05. D and E are the only models that had no levels of significance across any cycle. This means that larval age is not having any significant effect on In Vivo Exsheathment or Hatchability in any cycle. To determine if cycle and larval age were a combined effect on the individual variables of on In Vivo Exsheathment or Hatchability other models were ran, model O and model Q, neither of which returned significant readings. Overall, from the all the models ran we could loosely draw the conclusion that Cycle 3, or Spring, had the most significant impact on the results of the assays ran.