ACT Summer Studentship

Final Report

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Introduction

For eight weeks during the summer, I worked on a project investigating the role of actin in refractile body fusion in *Eimeria* parasites as part of the RVC summer studentship. This project took place in the Pathobiology and Population Sciences department at the Hawkshead campus of the Royal Veterinary College and was conducted with the parasitology research group of Professor Fiona Tomley and Professor Damer Blake where I was under the supervision of Dr. Virginia Marugan-Hernandez. During my second year on the Animal Behaviour, Welfare and Ethics course, I enjoyed the parasitology module very much and was excited to be working on a project related to the field of parasitology.

The project covered several aspects including the passage of cells to ultimately preparing a monolayer for infection, the harvest and sporulation of oocysts, and the purification of sporozoites to infect cells. Moreover, once the cells were infected, actin inhibitors were applied in order to investigate the effect this would have on refractile body fusion, the key aspect of my project.

Learning Outcomes

The learning outcomes of my project were:

- Describe *Eimeria* life cycle and recognise the different stages, and relate those with the different experiments performed in the laboratory
- Participate in the experimental design of the placement
- Accomplish calculation for preparation of reagents, cells and parasites
- Perform parasite infection in sterile conditions
- Analyse experimental data and perform statistical analysis
- Summarise a present data to a small audience
- Reflect about the placement experience

General achievements

Through this project, I was able to acquire many practical skills, most of which I did not have the opportunity to acquire in practical classes through my degree.

Firstly, I learned how to perform cell passaging and how to count cells using a Fuchs Rosenthal chamber to do calculations to determine which flask to grow the cells in or to prepare accurate number of cells for infection. It was my first time working in a sterile biological safety cabinet and using electric pipettes. Working in such an environment taught me to be cautious of contamination, such as placing lids of tubes upward and changing pipette tips if it has touched something other than the solution you are working with.

Secondly, I learned the whole cycle of infecting chickens with *Eimeria tenella* to obtain oocysts until purifying sporozoites to infect cells. I had to count the number of oocysts that we already had and calculate a dilution to prepare them at 4,000 oocysts per ml to dose the chickens. I was able to be present for the infection of chickens and after a week, we removed the ceca from the infected chickens to harvest the oocysts to prepare for sporulation. I learned how to carefully cut open the ceca and the techniques used to remove as much of the mucosal layer as possible. Apart from this, I was given the

opportunity to observe the culling procedure and dissection of chicken as well as lesion scoring to diagnose for coccidiosis. Here I was able to appreciate the importance of animal welfare, where culling was performed in a manner to cause minimum suffering. To prepare the harvested oocysts for sporulation, I was required to calculate dilutions of potassium dichromate and by this step, I was comfortable with calculating dilutions. Prior to cracking and hatching the oocysts and purifying the sporozoites, I was required to prepare stocks of phosphate-saline buffer and this was useful since it is already prepared for practical classes through my degree and therefore, I never knew how it was made. I followed the protocol that was given to me and I chose out the reagents from the lab and learned how to use magnetic stirrers and pH meters. I also learned how to prepare orders for reagents, where I was required to go through the materials of the protocol and check labs to identify any missing items. For any missing items, I had to search the Internet to find the cost and order codes for each item and input the details into an Excel spreadsheet.

Finally, once I had prepared a 24-well plate with MDBK cells, the oocysts were cracked and hatched and the sporozoites were purified through a washing column. Here I needed to calculate the number of sporozoites in the total volume to prepare them to an appropriate volume for infecting cells. After the cells were infected with sporozoites, I added the actin inhibiting drugs and finally fixed them for staining. To prepare the stock as well as the working solution for the drugs, I was required to calculate this using the molarity of each drug listed on the Internet. During this experiment, I learned several pipetting techniques such as pipetting gently across the well plate wall to avoid disturbing the cell monolayer and pipetting small volumes (5 µl) without touching the well plate. Once staining was completed, I fixed the glass coverslips onto glass slides for observation under a microscope. We also had the opportunity to look at our samples under the confocal microscope at the Camden campus and take pictures of our samples. I counted the merged and non-merged refractile bodies from all experiments using ImageJ software and analysed my data using Graphpad Prism. I also prepared a presentation of my experiment and results to present in front of the research group on my final week of the studentship.

Scientific Achievements (Results)

My experiment showed that Cytochalasin D at a 1 µM concentration significantly decreased refractile body mergence and across three experiments, 83.7% to 89.9% of anterior refractile bodies were still present (Figure 1). While Latrunculin A only inhibits host actin, Cytochalasin D inhibits host as well as parasite actin. The significant decrease in mergence between 500nM Latrunculin A and 1 µM Cytochalasin D suggests that refractile body mergence is greatly affected by disrupting sporozoite actin ($p = <0.0001$, Fisher's exact test).

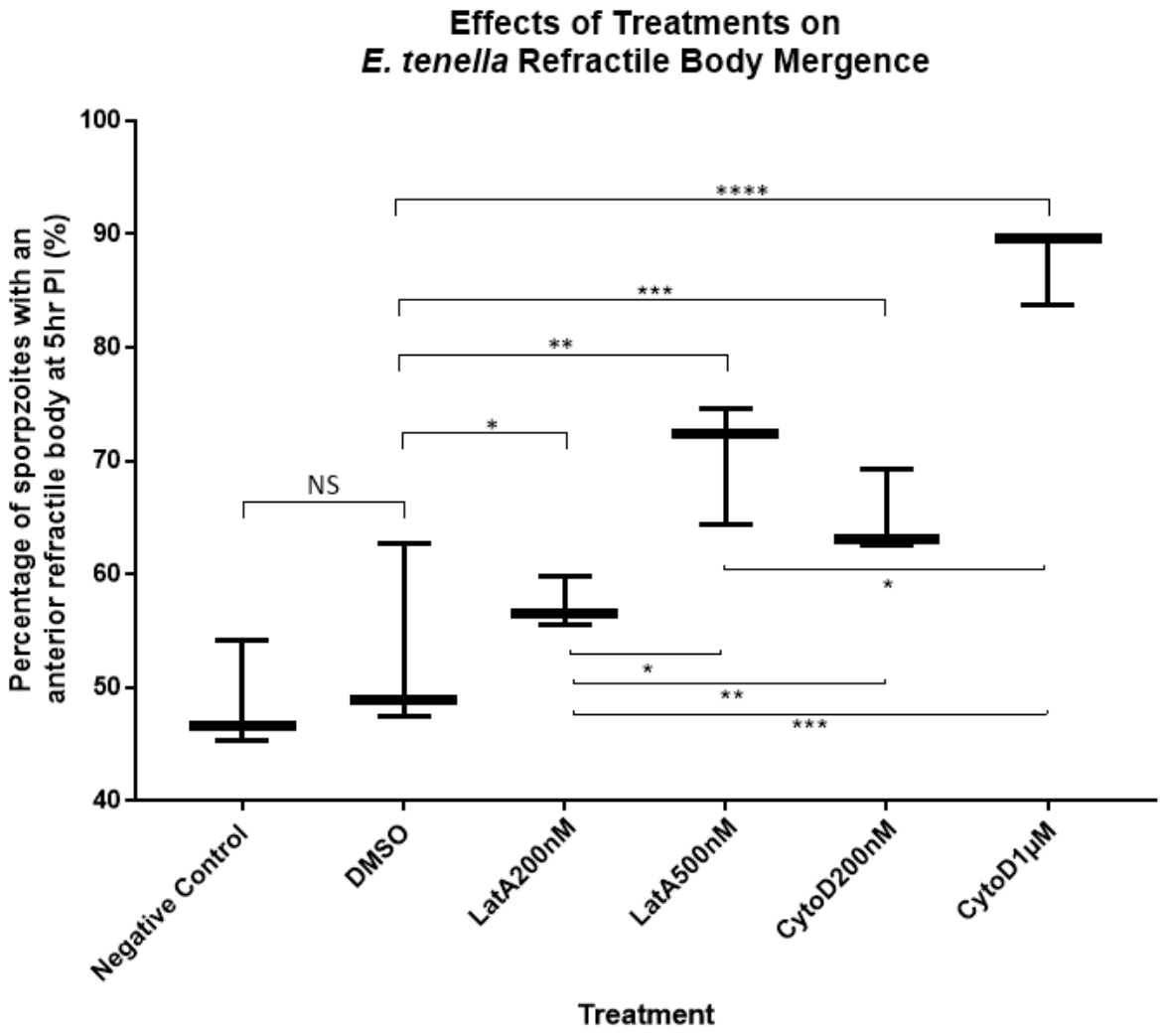

Figure 1- Effects of different treatments of sporozoites on refractile body mergence.

MDBK host cells were infected with *E. tenella* sporozoites and incubated in the presence of DMSO, 200 nM Latrunculin A, 500 nM Latunculin A, 200 nM Cytochalasin D, and 1 µM Cytochalasin D. Sporozoites treated with 200 nM Latrunculin A, 500 nM Latunculin A, 200 nM Cytochalasin D, and 1 µM Cytochalasin D contained a significantly higher percentage of sporozoites with an anterior refractile body compared to that of the DMSO control (*=p 0.0117, **= p<0.0001, ***= p<0.0001, ****= p<0.0001 - Fisher's exact test). Treatment with 500 nM Latrunculin A, 200 nM Cytochalasin D, 1 µM Cytochalasin D resulted in significantly greater percentage of sporozoites with anterior refractile bodies comapred to that treated with 200 nM Cytochalasin D (*= p<0.0001, **= p<0.0001, ***= p<0.0001 - Fisher's exact test). Treatment with 500 nM Latrunculin A resulted in a significantly lower percentage of sporozoites with an anterior fractile body compared to those treated with 1µM Cytochalasin D (*= p<0.0001).

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Reflection

Throughout this studentship, I not only acquired a lot of new skills, but I was able to learn what it is like to work in a research lab environment. As a second-year undergraduate student I did not have a great amount of practical skills, especially those used in parasitology research but my supervisor carefully and patiently taught me every step from passing cells, harvesting oocyst and purifying sporozoites, until infecting cells on a well plate to fix and stain samples for microscope observation. Once I got enough experience passing cells and purifying sporozoites, my supervisor allowed me to do many things on my own which gave me a sense of independence going into my project. By the third experiment of my project I was able to do most steps on my own such as infecting the cells with sporozoites, applying the drugs and staining samples as well as preparing them for microscopy.

I got to share an office with two PhD students which gave me a vague idea what it is like doing a PhD. For example, they were involved in many different projects of other people in the research group that did not necessarily relate to their PhD project. I also got to learn the different duties of people in the research group not directly related to their research such as organizing lab meetings and placing orders for reagents for other people in the research group. These things gave me a clearer idea of what it would be like if I decided to work in a research institute and got me thinking if this could potentially be a career field for me.