

Summer Research Project Application, Summer 2016

Section 1: Personnel

Faculty supervisor: Amanda Rumore, Assistant Professor, Department of Biology

Student participants: Di Bei, Biology and Chemistry, Class of 2018 and Callan Frye
Biology and French, Class of 2017

Section 2: Project title

In vivo evaluation of the over-the-counter treatments for equine streptothricosis.

Section 3: Abstract

Streptothricosis, also known as “rain rot” or dermatophilosis, is a common, self-resolving skin infection of horses, sheep and cattle. It is characterized by raised tufts of hair over coalescing, pustular scabs. The condition normally has no long-term health effects, but severe cases are associated with significant morbidity and mortality. Streptothricosis is caused by the actinobacterium *Dermatophilus congolensis*. In the equine industry, treatment usually involves topical over-the-counter products or household remedies that are used as on- or off-label. Little research has been done to confirm their effectiveness *in vivo*. This work will involve sampling and identification of *D.congolensis* infection from local equines, including strain ID and overall microbial burden. We will work with our research collaborators to determine the susceptibility of the strain to common treatments. We will then test the efficacy of the four common treatments through an *in vivo* analysis against an active equine *D.congolensis* infection.

Section 4: Project description

BACKGROUND

Streptothricosis is caused by the bacterium *D. congolensis* (Moriello, 2013). It most frequently affects agricultural livestock such as cattle, horses, and sheep. Humans are infrequently affected but cases have been reported after contact with infected animals (Burd, 2007). Most animals spontaneously recover from streptothricosis within 3 weeks with no long term health consequences, but severe cases can cause loss of mobility or prehension (Moriello, 2013). Chronic infection leads to significant hair loss and lesions can become secondarily infected or infested by fly larvae. In the most severe cases, death may occur. Risk factors for streptothricosis include prolonged exposure to rain, high humidity, and high temperature; infestation by certain ectoparasites; and immune system deficiency (Moriello, 2013). Age, sex, and breed do not appear to affect host susceptibility to this condition. The disease is characterized by skin lesions that progress through three stages: 1) matted, raised tufts of hair that pull off in clumps (i.e. “paint brush” lesions), 2) coalescence of lesions into scabs, and 3) accumulation of keratinized material at the site of the scabs (Moriello, 2013)

D. congolensis, the etiological agent of streptothricosis, is a gram-positive, facultative actinomycete that forms small, yellow “cake crumb” or umbonate colonies when grown on blood agar (Gordon, 1964). Like other actinomycetes, it has a high G+C genome; forms filamentous, highly branched hyphae terminating in coccoid cells that mature into motile zoospores; and is found in soil (Bida and Dennis, 1977). *D. congolensis* can lay dormant in the host epidermis when environmental conditions are unfavorable to the

bacterium. Upon exposure to high moisture, the bacterium releases zoospores that form new foci of infection or spread to other host animals. The zoospores germinate and hyphae penetrate the epidermis, leading to an acute inflammatory response and disease (Moriello, 2013).

Treatment for streptothricosis includes antibiotic therapy and/or topical application of lime sulfur or antibacterial shampoo (Moriello, 2013). Certain over-the-counter products are also used to treat the condition, including Banixx®, Shapley's M-T-G, Listerine®, Gold Bond® powder, Calm Coat®, Muck Itch® Skin Saver, Pete's Equine Remedy, Grape Balm Herbal Wound Healer™, tea tree oil, and essential oils (Ecoequine, 2013). The efficacy of these over-the-counter products is not fully understood.

In order to collect *D.congolensis* isolates for *in vitro* analysis and test the effectiveness of various treatments *in vivo*, we will utilize equine research subjects from Brook Hill Farm (BHF) in Forest, VA. BHF is a 501(c)(3) organization focused on rehabilitating unwanted horses for use in their therapeutic riding program. They are an accredited animal sanctuary through the Global Federation of Animal Sanctuaries (GFAS). Horses are kept in a common pasture that is subject to typical southern-Virginia summer weather conditions such as high humidity and frequent rain. Many of the horses are also at risk for *D.congolensis* infection due to shared pasture space and frequent minor skin injuries, a natural consequence of living in a 24/7 pasture habitat. BHF welcomes our group to assist them in determining the most effective treatment for their frequent "rain rot" infections during the hot, humid summer months.

EXPERIMENTAL DESIGN

This project will feature three experimental components:

- 1) Isolation and identification of *D.congolensis* from horses at BHF
- 2) *In vivo* treatment of active *D.congolensis* infection in BHF horses; therapeutic strategy will be determined from the results of the *in vitro* assays

Isolation of D. congolensis from horses

Brook Hill Farm will provide us with four horses for this experiment. Predicted *D.congolensis* infection will be visually identified by the project veterinarian. Horse isolates will be obtained by combining the methods of Haalstra (1965) and Burd et al. (2007). In summary, cutaneous swabs or streptothricosis scabs will be placed in a small bottle containing 1 mL of sterile water and allowed to stand for four hours. The bottle will then be opened and placed in a candle jar for 15 minutes. This promotes the release of zoospores by *D. congolensis* in the sample. An inoculating loop full of surface liquid will be plated on blood agar containing 1000 units/ml of polymyxin B and incubated overnight at 35°C in a capnophilic atmosphere (5% CO₂). Beta-hemolytic, yellow, hard colonies with an irregular margin that adhere to the agar and have a depressed periphery are suspected *D. congolensis*. A representative colony with this phenotype will be gram stained and tested for catalase and urease activity. Colonies that are: 1) gram-positive coccoid or filaments with transverse septa, 2) catalase positive, and 3) urease positive are presumptive *D. congolensis*. This work will be done in conjunction with our collaborators (Houlihan and Bei). Our collaborators will also use a more stringent identification by testing presumptive positive colonies PCR using the *D. congolensis agac* gene-specific primer set designed by Garcia et al. (2013).

Enumeration of D. congolensis on horses

Equine *D. congolensis* carriage will be determined by logarithmically diluting (1:10) zoospores (obtained using the method described above) through a series of test tubes containing 0.85% NaCl. 0.1 mL from each dilution tube will be spread on the surface of a blood agar plate and the plates incubated overnight

at 35°C in a capnophilic atmosphere (5% CO₂). Colonies with the phenotypic characteristics of *D. congolensis* will be counted and colony forming units per mL (cfu/mL) will be calculated by multiplying the number of colonies on each plate by the reciprocal of the dilution factor and averaging the results. Plates will only be counted if they contain between 30 and 300 discrete colonies.

Examining the effectiveness of therapies for D.congolensis infection in vivo

The effectiveness of four over the counter treatments will be investigated. The treatments will be chosen based on the results of the *in vitro* antimicrobial susceptibility tests completed by the Houlihan SRP group. We will utilize the four horses from which the initial isolates were obtained. We will purchase a bucket, sponge, and brush for each horse to eliminate the potential for cross-contamination between common grooming supplies. The project veterinarian will visually assess and stage the infection prior to the first treatment application and 2 – 4 days after completion of the final treatment. Photographic images will also be taken before, during (daily) and after, completion of the treatment regime. Treatments will be applied to the pasterns of the four legs of the horse per manufacturer or veterinary instruction. Each leg of each horse will be assigned a different treatment (ex: right-front = treatment #1, left-hind = treatment #2, etc) and location will be rotated among the four horses so that each treatment is applied at each of the four leg positions. Treatments will be applied daily for 5 – 10 days with daily visual assessment and enumeration following the above protocol. Overall effectiveness will be evaluated via veterinary analysis, enumeration data, and visual improvement of the horse's coat.

SIGNIFICANCE

This work will provide useful information about the effectiveness of over-the-counter remedies for streptothricosis, a common cutaneous infection in horses. This information will be specifically useful to local agriculturalists in the treatment of “rain rot” in their livestock. Furthermore, although *D.congolensis* infections rarely cause long-term health effects and are generally self-limiting, little research has been done on its treatment despite its impact including economic loss and required veterinary treatment in the cases of secondary infection. This project can be reasonably completed in eight weeks. Positive results may encourage continuation of this project as an Independent Study during the 2016-17 academic year. No course release option is requested for continuation of this project.

CITATIONS

Bida SA and Dennis SM. 1977. Sequential pathological changes in natural and experimental dermatophilosis in Dunaji cattle. *Research in Veterinary Science* 22:18-22.

Burd EM, Juzych LA, Rudrik JT and Habib F. 2007. Pustular Dermatitis Caused by *Dermatophilus congolensis*. *Journal of Clinical Microbiology* 45(5):1655-1658.

Ecoequine. 2013. Rain Rot Remedies for Your Horse [Internet]. [Cited 22 Feb 2016.] Available from <https://ecoequine.wordpress.com/2013/10/14/rain-rot-remedies/>

Garcia A, et al. 2013. Development of a real-time SYBR Green PCR assay for the rapid detection of *Dermatophilus congolensis*. *Journal of Veterinary Science* 14(4):491–494.

Gordon MA. 1964. The Genus *Dermatophilus*. *Journal of Bacteriology* 88(2):509-522.

Haalstra RT. 1965. Isolation of *Dermatophilus congolensis* from skin lesions in the diagnosis of streptothricosis. *Veterinary Record* 77:824–825.

Moriello K.A. 2013. Overview of Dermatophilosis [Internet]. The Merck Veterinary Manual. [Cited 22 Feb 2016.] Available from http://www.merckvetmanual.com/mvm/integumentary_system/dermatophilosis/overview_of_dermatophilosis.html

Section 5: Dissemination goals

Callan and Jessica will present this work with our collaborators at the 2016 MARCUS conference. The results of this project will be appropriate for presentation at the 2017 Equine Science Society (ESS) conference and possibly manuscript submission to the Journal of Equine Veterinary Science.

Section 6: Past SRP outcomes

Summer 2015: I served as faculty mentor on two projects during SRP 2015. The first included two students, Olivia Reed ('16, BIOL) and Tetiana Poliakova ('18, BIOL). The two students carried out cellular assays where they measured the entry and localization of the fungal allergen, Alt a 1. This involved a considerable amount of experimental design and learning the technique of immunofluorescence. They also gained valuable experiences with microscopy. Olivia was awarded a VFIC grant towards this work. Olivia and Tetiana presented at the 2015 MARCUS conference and received an SRP Travel Grant to present at the Eastern Sciences Conferences this coming April. Both students have continued the project during the Fall 2015 and Spring 2016 semesters as Independent Study projects. The second project included one student, MacKenzi Brown ('18, BIOL). In this project MacKenzi used equine heart-rate monitors to determine the ideal "warm-up" and "cool-down" time for the horses at the Randolph College Riding Center. This entailed daily rides of four mounts with concurrent recording of heart rate and video recording of riding sessions. MacKenzi is completing the statistical analysis of this data during the Spring 2016 semester as an Independent Study. She presented at the 2015 MARCUS conference and plans to submit a manuscript once final results are compiled.

Summer 2014: Hart Gillespie ('15, Math and Physics), Thao Nguyen ('17, Undeclared), and Shaun Chopp ('15, Biology) participated as student researchers on the SCHEV-funded grant to Peter Sheldon, Peggy Schimmoeller, and Amanda Rumore entitled "Science and Math Links: Research-Based Teaching Institute". Although the students were from different disciplines, they each showed enthusiasm towards learning about educational practices. They also received hands on teaching experience by leading a Science Camp at the Jubilee Child Development Center. The students presented at MARCUS in fall 2014 and have been asked to apply to present at 2015 Symposium of Artists and Scholars. In addition, Thao Nguyen and Hart Gillespie presented the results at the American Association of Physics Teachers (AAPT) conference in January 2015 through a SRP Travel Grant Award. *The SCHEV grant was not funded for 2014-15 however it has been funded for the 2015-16 cycle.*

Summer 2013: Sydney Henson ('14, Education and Spanish, '15 Special Education) and Katherine Lesnak ('15, Political Science) participated as student researchers on the SCHEV-funded grant to Peter Sheldon, Peggy Schimmoeller, and Amanda Rumore entitled "Science and Math Links: Research-Based Teaching Institute". Sydney and Katherine assisted us with a weeklong Teaching Institute for local K-6 teachers while learning how to develop problem based learning activities. They also received hands on teaching experience by leading a Science Camp at the Jubilee Child Development Center. Both students spent time completing extensive literature reviews in preparation for their senior papers. They also compiled, input, and analyzed data collected from the teachers and students in the previous (2012) study. They presented the outcomes of the 2012 study at the Virginia Educational Research Foundation (VERA)

conference, MARCUS, and the Symposium of Artists and Scholars. In addition, Peter Sheldon presented the results at the American Association of Physics Teachers (AAPT) conference in January 2014. *The grant was again submitted and funded by SCHEV for 2013-14.*

Section 7: External funding

No external funding available.

Section 8: Course release

No course release is being requested.

Section 9: Academic credit

Callan Frye would like to receive 3 academic credits.

Section 10: Budgetary needs

Horse Expense (4 horses x \$75ea) Brook Hill Farm	\$300
Veterinarian Evaluation Fee (2 clinical evaluations and IACUC review) Dr. Ronald Fessler or Dr. Martha Moses	\$150
Grooming Supplies (bucket, sponge, brush X 4) Western Ways	\$50 (estimated)
Over-the-counter treatments (ex: Bannixx ®, Listerine ®, Shapley's M-T-G ®, zinc oxide, Novasan) Western Ways or Dover Saddlery	\$100 (estimate)
Mileage to/from Brook Hill Farm (31mi round trip x 15 trips = 465mi total) 465mi x \$0.51/mil	\$237.15
Miscellaneous veterinary supplies (VetWrap, gauze, etc.) Western Ways	\$50
Bacterial growth medium (BHI broth and agar, blood agar, etc.) VWR	\$0.00 (collaborators)
Consumables (Tubes, paper discs, Petri plates, pipette tips, etc.) VWR	\$0.00 (collaborators)
Total expenses	\$ 887.15

JUSTIFICATION: We will require the use of the equine subjects to confirm the results of our *in vitro* studies (\$300). Since this will require IACUC approval, federal law mandates that veterinary oversight exists from initial consult through the duration of the project (\$150). We will have to make multiple day trips to BHF to collect samples and complete the treatment protocol (\$237.15). Equine and veterinary

supplies, including the treatments, will be purchased from commercial vendors (\$200). Consumable laboratory supplies and bacterial growth medium will be provided by our collaborators.

Section 11: IRB or animal research

IACUC approval will be required for this project and obtained prior to the start of SRP.

Section 12: Statement about student researchers

As faculty mentor, I will work side-by-side with Callan and Di while conducting all experiments. I anticipate spending 15 to 40 hours mentoring them each week. The goal of this close supervision is two-fold: 1) direct mentorship will maximize student learning, and 2) it will enhance productivity so we can gather enough data to publish a high-quality paper describing our findings. This project spans multiple disciplines, including microbiology and equine science. This breadth will facilitate an exceptional learning opportunity.

I have known Di since fall 2014 and Callan since spring 2015. Both students are serious, enthusiastic, and inquisitive students who display a natural curiosity for the sciences. Their attention to detail, capacity for independent thought, and verbal and written communication skills are all well above average when compared with their peers at Randolph College.

The students have a solid set of laboratory skills, good lab sense, and are exceptionally well-organized. Callan and Di have completed 300-level coursework in Microbiology and/or Immunology and their academic records will serve as important foundational knowledge towards this project.

The students will be responsible for all aspects of the project including the samples, microbial cultures, and experimental design for examining the various treatments. I will expect them to maintain a detailed laboratory notebook and utilize DropBox for storage of data and images.

I will facilitate the relationship with Brook Hill Farm and use of their horses as our research subjects. Accordingly, I will oversee submission of all IACUC documentation and IACUC training for the student researchers. The students will be responsible for experimental design, sample collection, microbial identification, *in vitro* testing, and *in vivo* testing of treatments.

Section 13: Student Statements

Statement from Callan Frye

As a student of biology with strong interests in disease, microbiology, and immunology, I am intrigued by this project because it very directly relates to working with a microorganism that causes disease and finding effectual methods of treating said disease. This project also interests me because it features both lab work and field work. I enjoy working in the field and in the out-of-doors, so I'm excited to contribute to a project that allows for such. More importantly, however, I aim to one day work in a lab setting doing exceedingly similar research to that of this project, so I am elated to garner valuable experience in this regard. I believe that this experience will very directly contribute to beginning my chosen career path as a researcher and academic. This incredibly applicable experience is the primary asset I wish to gain from our Summer Research Program via this particular project. I am also looking forward to applying many of the principles of bacterial identification and isolation, and methods of determining the effectiveness of

antibiotic compounds that I have learned in the classroom and the lab. This application will reinforce these concepts and methods which are vital to success in my field.

There are several ways by which I believe I am capable of adding to this research. I have experience working in a lab setting— specifically in relation to microorganisms— and have completed coursework in microbiology, which is central to this project. I also have experience doing extensive literary research in biology, which has cultivated research skills that will certainly benefit the project in that I will be able to effectively sift through information and find relevant publications for additional background research. I also work in a relatively interdisciplinary fashion (I have moderate experience in the fine arts and languages), and may thus offer a unique perspective on our work. Additionally, I can contribute to this project a strong work ethic, easy adaptability, and well-practiced presentation skills

Statement from Di Bei

My name is Di Bei. I am currently working towards a double major in chemistry and biology at Randolph College. Upon graduation, I intend to apply for MD/PhD and become a research physician.

Since I entered the college, I've had the pleasure of having Dr. Houlihan as my instructor. I've also taken Dr. Rumore's Immunology class. Dr. Rumore and Dr. Houlihan have offered me a lot of help and advice, and I look forward to the opportunity to work with them very much.

As I have a deep-rooted interest for chemical and biological studies, SRP highly attracts me. Dr. Rumore and Dr. Houlihan's biology research requires in-vitro isolation and testing of the microbe and the in-vivo sample collection and testing of treatments. I believe I will obtain a lot of useful knowledge and valuable lab experience throughout this research.

In applying to Randolph Summer research program, I recognize that it will offer me a precious opportunity to perform hands-on research in lab with an outstanding professor. This program will prepare me for my medicine studies in the long run and reveal paths I might otherwise never discover. As an international student and a college sophomore, I still have a long way to go before eventually achieving my goal and making substantial breakthroughs in medicine. However, SRP will help me stretch out my arms farther for my dream, and I will cherish this chance very much.