Specific Aims

Hot shoeing is a process in which a hot metal shoe is burned onto an equine hoof. This process became popular in the 16th century with the thought that the shoe then fits the foot better than applying the shoe cold can fit. (1) Since this original thought and the onset of germ theory there is anecdotal evidence that hot shoeing can kill microbes on the hoof capsule surface as well as “seal the tubules” to prevent further microbe intrusion into the hoof capsule. (2,3) Although farriers have fallen on these arguments time and again there has been no scientific record of these claims to the authors knowledge. Proving this old adage true could help the farrier industry validate their practices with clients and veterinarians and help them do the best they can for equines.

Some microbes have the ability to live at high temperatures, but these microbes are better suited to hot springs than to soil that equines are likely to walk on. Burning shoes on at temperatures of 750°F should kill most microbes on the foot surface as evidenced by these studies. (4,5,6) Hoof tubules make up horn tissue that forms the hoof wall and are arranged as a bunch of vertical “straws” that are held together by extracellular matrix proteins like proteoglycans. These tubules can be opened by trimming down the hoof. I hypothesize that hot shoeing hoof capsules with a shoe at 750°F temperature for 5 seconds will kill any microbes found on that surface of the hoof capsule. I further hypothesize that this act will also close the tubules in the hoof capsule, further protecting it from invading microbes.

Aim 1: Elucidate the role heat plays in killing microbes on the solar surface of the hoof capsule.
Heat has been shown to be an effective way to kill many microbes. (4,5,6) Until now there has been no work on elucidating these claims when it comes to hot shoeing an equine foot. A sterile swab will be used to take a sample before a trim, and after a trim, and after hot shoeing on the left hoof on the solar surface of the foot compared to a cold shod foot on the contralateral limb. I hypothesize that the effect of hot shoeing will be to kill microbes on the solar surface of the foot where the shoe was applied.

Aim 2: Determine whether hot shoeing does seal the tubules that make up hoof wall and whether this process does prevent microbes from entering the tubules.
Since another touted benefit of horseshoeing is “sealing the tubules” of the hoof wall to prevent microbial invasions (2,3), and no experimentation has been done to prove this, I propose measuring the effect of hot shoeing on sealing tubules. Horses will have one foot hot shod, and the contralateral limb cold shod. A clipping from a sterile knife pass before and after hot shoeing the hoof will be histologically processed to access tubular closure and microbial invasion. I hypothesize that hot shoeing will seal tubules that make up the hoof wall and that hoof capsules that are hot shod will have less microbial activity in them than cold shod hoof capsules.
RESEARCH STRATEGY

A. Significance:
Horses have been shod with metal since the 6th and 7th centuries, and by 1000AD it was a mainstream practice to shoe horses with iron. Hot-shoeing, or the practice of placing a hot, fitted shoe on the hoof before nailing it on, became popular in France and Britain in the 16th century.\(^{(1)}\) The benefits of cold versus hot shoeing have been a debate for centuries, and there has been no resolving which method is superior. Gaining more knowledge of the attributes of each style of shoeing can help farriers decide what is best for the horses they serve.

Common problems in the farrier industry are microbial pathologies such as thrush, canker, and white line disease. Thrush has been shown to be a bacterial problem, although fungi can play a role in chronic cases. It is characterized by a noxious tar-like substance on a hoof. It is commonly found in the commissures of the frog, on the frog, especially in the central sulcus, and can even grow in the sole. Thrush in itself is not considered a lameness, however if it eats away at enough horny tissues in the foot it can invade sensitive structures causing soreness.\(^{(7,10,11)}\) Canker is a disease in which there is uncontrolled and abnormal growth of the frog tissue caused by mitogens released from a microbial offender that stimulate uncontrolled growth. Although there is much unknown about canker, it can be traced to unclean living conditions.\(^{(8,10,11)}\) White line disease is also referred to as “seedy toe” and is a fungal infection in which bacteria may be involved that attacks the inner zone of the hoof wall. The treatment for white line disease is very invasive requiring farriers to resect the hoof wall, and recovery is slow.\(^{(9,10,11)}\)

In an internet search, reasons for hot shoeing include eliminating bacteria, sealing horn tubules, stabilizing a shoe with clips, and a better fit between the hoof surface of the shoe and the hoof.\(^{(2,3)}\) It was impossible to find studies that support these claims however; so we would like to investigate the eliminating bacteria and sealing the hoof tubules hypotheses. Both these assertions have been linked in statements about preventing microbial pathologies such as thrush and white line diseases. Both thrush and white line diseases are common pathologies that can be challenging to treat, especially if the horse becomes prone to these infections. Confirming, or not, that hot shoeing can help manage these conditions would have a large impact on the farrier industry.

B. Approach:

Aim 1: Elucidate the influence of hot shoeing on the bacterial population on the solar surface of the foot.

Aim 1 Rationale:
The bacterial and fungal content on the bottom of the foot has been blamed for many microbial pathologies such as thrush white line disease and canker. Logically, reducing the microbial population on the solar surface of the foot, especially at the white line would be a logical approach to reducing the incidence of these pathologies. Many sterilization techniques utilize heat to ensure an aseptic environment.\(^{(4,5,6)}\) We decided to test how effectively hot shoes sterilize the foot.

Aim 1 Experimental Design:

**Experiment 1A: Placing a hot shoe on a foot to reduce the bacterial population.** As previously discussed, There is evidence that heat can kill bacteria. I hypothesize that placing a hot shoe on the solar surface of the foot will decrease the bacterial population on the seared area of the foot.

To intricately dissect this issue, 10 horses will be chosen that have at least a year history of being hot shod. This ensures they are used to the procedure, eliminating the possibility of stress, and gives us a hoof capsule that has been shod the same way throughout the hoof wall. Hoof wall grows out about a centimeter a month, so most horses will have replaced their hoof wall every year. I will take a sterile swab and take a sample swab of the foot after it has been picked out on both left and right forefeet. I will then trim both feet, taking another sterile swab of both feet in the same place before putting the foot on the ground. I will then proceed to shoe the left fore cold. For the right fore I will heat up the fitted shoe until an orange color. The temperature...
will be monitored with a laser operated thermometer until it cools to 750°F. The shoe will then be placed on the foot for 5 seconds, and a third sterile swab on this foot will be used in the same place as before the trim, and after the trim. The swab samples will be sent to the University of Iowa’s microbiology lab and cultured. The lab will count the number of colonies in the cultures. This process will be repeated for each of the 10 horses every 6 weeks for 6 months.

Aim 1 Expected Outcomes and Alternative Approaches: We expect that the bacterial counts will be reduced in the samples taken after hot shoeing compared to the samples taken after the trim and the samples taken at baseline. I foresee no difficulty in carrying out these methods. I am an experienced farrier that has hot shod many cases, and I have extensive experience using aseptic technique which is require for proper bacterial sampling. However, if samples persistently come up negative for bacteria we can try different culture methods that utilize different nutrients in the agar. If we persistently come up with too many bacterial colonies to count, we can dilute our samples 1:100 and re-plate the dilution. If there is a persistent shoeing issue I will have another farrier perform the work.

Aim 2: Determine whether hot shoeing seals tubules in the hoof wall.

Aim 2 Rationale:
It is a common notion that the tubules of the hoof wall can be sealed with heat. Although this notion is repeated in various horse world forums, farrier and client alike, there is absolutely no evidence to support this claim. If it is true that heat seals the tubules of the hoof wall further study could be done to elucidate the benefits or detriments to this action. **We hypothesize that hot shoeing seals hoof wall tubules.**

Aim 2 Experimental Design:
**Experiment 2A: Hot shoeing can seal hoof wall tubules.**

The hoof wall is made of tubules that grow from the proximal hoof capsule at the coronary band and run parallel to the laminae down to the ground bearing surface of the foot. These tubules are held in place by perpendicular tubules known as intertubular horn. White line disease is an example of an infection of the hoof wall tissue. It has been suggested that the infection starts in weak hoof wall to laminar interface and insinuated that bacteria and fungi can enter this area via hoof wall tubules. Although one could imagine logical reasons for the benefits of sealing the ends of hoof wall tubules there is no literature supporting that this happens when a hot shoe is applied. Here we seek to understand the relationship between heat and the hoof wall tubules. **I hypothesize that heat sears the hoof wall tubules and thus, the tubules are closed.**

To investigate, I will make a shaving with a sterile hoof knife that encompasses hoof wall, white line and sole from the toe region of the foot right after cleaning the foot, after trimming the foot and before and after hot shoeing the right fore and from the left fore after cleaning the foot and after trimming the foot. This will be performed after taking the swab sample off of the same 10 horses, and will be repeated every 6 weeks for 6 months. This shaving will be thin to allow for the multiple sampling. These shavings will be prepared to send to the University of Iowa’s histology lab where they will be mounted on a slide and stained using a standard H&E protocol. This protocol stains nucleic acids purple, and proteins pink, allowing us to view the tubules under the microscope. We should be able to observe a sealed tubule and count the number of tubules that remain open relative to the number that are seared shut. We will further stain these slides with a gram stain to observe at the number of bacteria in the shaving sample. Gram staining will allow us to visualize gram positive bacteria separately from gram negative bacteria and allow us to assess the numbers of bacteria in and around the tubules with and without hot shoeing.

Aim 2 Expected Outcomes and Alternative Approaches: I expect that the tubules will sear resulting the ends of the hoof wall tubules will be closed. As in aim1, I don’t expect any difficulties in this study because of my experience in farriery and aseptic technique. I may have to optimize the best size of shaving to use for histology. This can be remedied easily with a small experiment that mounts various sized shavings and stains them with the same H&E protocol.
References


2) Farmilo, D. "The Pros and Cons of Hot Shoeing." *David Farmilo*. Horse Farrier


7) O'Grady, S. "Thrush." *Equine Podiatry | Dr. Stephen O'Grady*, Veterinarians, Farriers, Books, Articles. Virginia Therapeutic Farriery


Plans to Share Research

**Science/Veterinary Community:** I plan to attend the University research day where I will share a poster with the veterinary community at the University. The poster will outline the project and report my results.

**Farrier Community:** I plan to write an article for The American Farrier’s Journal in laymen’s terms.
Facilities and Resources

This project requires a space with a flat concrete floor, a forge, an anvil, and a place to safely tie horses. My shop space has all these things and is 50ft by 30ft in size, giving plenty of space for organization and tables to use for collecting hoof clippings and aseptically handling sterile tools.

I will need an infrared thermometer laser gun to measure the high temperatures of the shoes in order to ensure a consistent measure of temperature throughout the experiment this has been purchased already. I will use the University pathology and histology lab services to process my samples.

Tools I have:
Rasps
Nippers
Hoof Knives(2)
Driving Hammer
Crease Nail Pullers
Pull-offs
Clinchers
**Budget**

**Shoeing supplies:**

10 horses, 20 shoes per cycle, 4 cycles leads to 80 shoes at $2.50 per shoe leads to a cost of $200

A rasp lasts 20-30 horses, use 25 as an average. 10 horses done 4 times is 40 rasp uses and 2 rasps. Rasps cost about $25 each leading to a cost of $50.

Nails are $0.07 each. I will need a minimum of 12 nails per horse per cycle, but we should factor 15 per horse per cycle for pulled nails. 150 nails per cycle with 4 cycles is 600 nails over the course of the study with a total cost of $42.

Since I will have to shoe these horses on a regular schedule anyway, I am willing to absorb these costs as a part of my business.

**Microbiology supplies:**

10 horses, 4 swabs per horse (before shoeing and after shoeing for each foot), 4 cycles is 160 sterile swabs. A box of 200 swabs is $12.

10 horses, 4 swabs per horse, 4 cycles is 160 tests. University of Iowa pathology lab charges $5 per analysis giving us a total of $800

**Histology Supplies:**

10 horses, 2 samples per horse (after trim but before hot shoeing and after hot shoe has been applied), 4 cycles is 80 slides, 80 50mL conicals. 50mL conicals are $0.73 a piece, 80 conicals cost $58.40 University of Iowa Histology lab charges $10 per slide giving us $800 in histology charges.

**Totals:**

- Total cost of study: $1904
- Costs I’m willing to absorb: $362.40
- Total asked from grant: $1600
Animal Welfare

1. Description of animals and how they will be used
Equus caballus, or horses will be the only species used in this study. Both limbs will be trimmed according to a standard trimming protocol with the same tools by the same person. The right limb will be hot shod with a shoe shaped to the foot at 750°F temperatures for 5 seconds. Chris Gregory’s text book recommends not exceeding about 4 seconds, so this would be maybe a little longer than the average amount of time a hot shoe is applied. The light knife pass on the toe section of the hoof wall will create a small clipping immediately after hot shoeing or completing the trim on the cold shod foot. The left limb will be shod with a shoe cold shaped to the foot. The horse will be shod in this fashion every 6 weeks for 6 months for a total of 4 shoeings.

2. Justifications for use of animals
There is no current model system that can be used for this study. Horses are unique in their athletic abilities that make them perfect for work. Many horses require the use of shoes to stay sound in their work. No smaller scale animal would be suitable due to the lack of hoof capsule needed to complete this study. The horse also needs to have access to normal environmental conditions to test the microbial conditions accurately, and not be held in a unique laboratory setting. We feel that this aspect of the study is beneficial to the horses in question.

3. Veterinary care
Dr. Doe will be on call if there is any indication of illness or stress or in case injury results while under our care. We do not anticipate the need for a veterinarian because these are very standard day to day procedure for equines.

4. Provisions to minimize discomfort, distress, pain and injury
The horses chosen will have to be hot shod for a year previous to the start date of the study. Therefore, all horses selected will have been exposed to the process of hot shoeing that can sometimes cause fear and anxiety in animals that have not been previously exposed. It has been stated that hot shoeing does not cause any physical pain or discomfort. If a horse is expressing fear, anxiety or any signs of pain or distress the veterinarian on call will be called immediately to handle pain or distress and the protocol will be halted for anxiety and fear and a different horse will be used in place of the distressed horse.

5. Euthanasia
Euthanasia is unnecessary in this study.
Mentor Versus Farrier Responsibilities

I believe Dr. Doe will be the best choice for a successful project. Dr. Doe attended a College of Veterinary Medicine followed by an internship and residency at a University. Dr. Doe specialized in equine medicine while at University, and her research experience includes studies on laminitis. Dr. Doe is currently a faculty member, associate professor of equine internal medicine, at another University. I believe that these experiences will benefit our project because Dr. Doe’s experiences have lent her a particular perspective on the equine hoof that some veterinarians don’t receive. Her research experience will help me with study design and execution.

Dr. Doe will be the veterinarian on call if we need one while shoeing the horses. She will also help me with scientific design, data analysis of my experiment, and help me with my publications. Dr. Doe will also help me gather the necessary microbiology tools and resources I will need to carry out the experiment.

I will be responsible for gathering the horses I need to complete the study, assisting in study design, carrying out the experiments, data analysis, and writing the final publication.
Cover Letter

To the AFA Research Committee,

I am applying for grant money that can be awarded by the American Farriers Association for studies that impacts the farrier industry and add to knowledge of the equine hoof, care of the equine hoof, pathologies of the equine hoof or biomechanics/anatomy pertaining to the equine hoof. The idea for my proposal is to prove that hot shoeing positively impacts the equine hoof by keeping the microbial population down and by sealing the hoof tubules that make up the hoof wall tissue. This project is important to the farrier industry because if these claims we have made for centuries are true, we can prove to the veterinary and client communities that we are practicing for the benefit of the horse. We can also use this practice specifically in some cases to decrease the incidence of some microbial pathologies in the equine hoof.

I believe that I am the right person to carry this project out because I have numerous experiences in the biological sciences. I got my bachelor’s degree at the University of New Hampshire in general biology in which I was required to take many classes that pertained to microbes. I have experience in research there as well studying the ovarian cycles of dairy cows and helping with a project that involved uterine infections in those cows. I furthered my research experience as a research assistant II at Boston Children’s Hospital where I gained valuable experience in keeping microbes at bay so that I could work in sterile environments. This experience lends me the skills I need to accurately assess what microbes come from the horse’s hoof capsule from those I may carry on me or my tools. My last research experience was at the University of Michigan where I started my PhD studies. Although I quit my PhD in 2016, I had completed and submitted an F32 grant to the NIH that gave me invaluable experience in study design and the scientific method.

Although I am relatively new to farriery, I have had several excellent and well respected farriers and veterinarians compliment me on my trim job and basic skills. I also am invested in continuing education in the form of contests and clinics, and I am pursuing AFA certification at this time. I am confident that my trimming protocol is consistent between horses and my experience in creating systems has helped me ensure consistency. I am sure that the experience of carrying this study out will further my systems and help me get better at hot shoeing and further our knowledge in the farrier community.

I thank you for taking the time to consider my research proposal and I hope that I can help the American Farrier’s Association bring more knowledge to the industry.

Sincerely,

Katelyn E. Panos
Education:
2014-present  **Student/Apprenticeships**
Oklahoma Horseshoeing School, Purcell, OK 8 weeks
- 6 month apprenticeship with Rick Howe, New Boston, NH, prior to school
- Apprenticeship with 3R Forge and Farriery, Dansville, MI, following school

2014-2016  **Graduate Student Researcher**
Michigan State University (MSU), East Lansing, MI
- Research Advisor Dr. Kurt Hankenson
- Studying the role of Notch signaling and lineage status of a cell in osteoblastogenesis.

May, 2011  **Bachelor of Science, General Biology**
University of New Hampshire (UNH), Durham, NH

Experience:
2014-present  **Farrier**
Self Employed, Lansing, MI
- Mentored by David Hallock DipWCF, FITS, CJF and Daniel Brown CF
- Trimmed and shod ~70 horses while a graduate student.
- Full time in July 2016, 3 years’ experience, 6 months full time.
- Passed CF written with 93%, passed shoe board 2016. Will retake live shoeing in 2017
- Several successful laminitis cases with and without veterinarians using radiographs and trimming to maintain comfort and improve condition of foot and position of coffin bone.

2011-2014  **Research Assistant I / Laboratory Manager**
Children’s Hospital Boston, Boston, MA
- General Lab duties included stocking the lab, ordering, and organization. Instrumental in starting the laboratory for Dr. Suneet Agarwal (June 2011).
- Cell culture, characterization, and care of iPES, fibroblasts, hES and mES cells.
- Diagnosed patients with genetic causes of Dyskeratosis Congenita.
- Studied Pearson’s Marrow-Pancreatic Syndrome using induced pluripotent stem cells.
- Assisted in multiple lab collaborations.

2008-2011  **Laboratory Assistant**
UNH Laboratory, Durham, NH
- Ultrasonography in cows to track ovarian cycles.
- Established primary endothelial cell cultures from the bovine corpus luteum.
- Immunohistochemistry using fluorescently labeled antibodies for endothelial cell markers.
- *In vivo* and *in vitro* ovarian follicular fluid collections, lutectomies and ovariectomies.

2007-2011  **Veterinary Technician**
Henniker Veterinary Hospital, Professional Association, Henniker, NH
- Assisted in examinations of numerous species.
- Diagnostic tests and radiograph processing.
- Performed a multitude of treatments.
- Receptionist and office work.
- Hospital maintenance

Technical Skills:
**Farriery**-
Simple hand mades  CF shoe modifications  Forge welding
Jump forge welding  Foot mapping  Anatomical knowledge
**Cell Culture**-
Passaging cell lines  Cryopreservation  Cell enumeration
Cell dissociation  Primary cultures  Cytotoxicity testing
Plant cultures  Stem cell cultures  Cloning
Growth curves  Karyology  Transformation
Organ culture  Virus production  Viability determinations
Reprogramming  MEF production  Blood/osteoblast differentiation assay
**General Laboratory Skills**

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**Animal Handling Skills**

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**Computer Skills**


**Societies, Licenses, and Presentations:**

**Publications**
- Cherry, ABC, **Gagne, Katelyn E** et al. "Induced Pluripotent Stem Cells with a Pathological Mitochondrial DNA Deletion." Stem Cells. 31 (2013): 1287-1297.

**Conferences and Awards**
- American Society for Hematology Conference, 2014
  - Pearson Syndrome in a Cohort of Diamond Blackfan Anemia Patients.
- ASH Abstract Achievement Award
- UNH Undergraduate Research Conference, 2011
  - Characterization of Endothelial Cells of the Bovine Corpus Luteum.

**Presidential Scholar**
- Dean's List 6 semesters.

**Alpha Chi Sigma, Professional Chemistry Fraternity**
- Mu Chapter, Master of Ceremonies

**Commercial Driver's License-B**

**Leadership and Community:**

**Bus Driver**
- Wildcat Transit, Durham, NH
  - Bus driver for students and general community, student and administration relations.

**Peer Leader**
- Peer Led Team Learning, Durham, NH
  - Led general chemistry students in a study group to develop study skills.

**Organic Chemistry Tutor**
- UNH Chemistry Department, Durham, NH
  - Organized program, attended class, and tutored students.

**Leader and Teacher**
- UNH Therapeutic Riding Program, Durham, NH
  - Taught people with disabilities how to ride horses and other equine skills to enhance their overall well being.

**Equine Related Jobs**
- Private Residence, Middleton, MA
  - Trained two draft horses for a client in dressage, trail, and basic manners.
- Windkist Equestrian Center, North Andover, MA
  - Assisted teaching horseback riding lessons and coaching the farm team in dressage and other disciplines. Trained lesson horses for optimal student performance.
- University of New Hampshire Equine Program, Durham, NH
  - Worked at the barns mucking stalls, caring for horses, completing morning turnout and supporting equine students.
References:
Name of reference
Phone number
Email address
(Work) Address