[EXTERNAL]

--- Today's Date ---

07/15/2025

--- Name of 501(c)(3) Organization ---

Shifting Strides

--- Federal Tax-Exempt ID# ---

99-0823421

--- Year Established ---

2024

--- Amount Requested ---

15000

--- Name of Executive Director ---

Alexis Bradley

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06455

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--- Email address ---

shiftingstrides24@gmail.com

--- Work Phone # ---

+18184547915

--- Organization's website ---

<https://shiftingstrides.net/>

--- Copy and paste the link to your organization’s most recent filed IRS Financial Statements (#990): ---

<https://app.candid.org/profile/15435592/shifting-strides-inc-99-0823421>

--- Upload all supporting documents required for your application and your organization’s most recent filed IRS Financial Statements (#990): ---

<https://www.terfusa.org/wp-content/uploads/wpforms/809-a07c4cb3463c1ac16da216b7fda5277e/990-8d09f21b1f3a6d9237b9c9a8505a42e6.docx>

<https://www.terfusa.org/wp-content/uploads/wpforms/809-a07c4cb3463c1ac16da216b7fda5277e/Battison_CV_April2025-ed375760a5de4e71dd0d3ccc4edbd23f.docx>

--- Farm/Facility Name ---

Shifting Strides

--- Farm/Facility Physical Location (City, State, Zip) ---

Ellington, CT, 06029

--- Farm/Facility Mailing Address ---

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--- 1. Brief mission statement and describe the distinguishing features of your organization that supports the mission of TERF and the relevance to this proposal. ---

The mission of Shifting Strides, a 501(c)(3) nonprofit dedicated to rehoming and retraining off-track racehorses for successful second careers, aligns closely with the goals of the Thoroughbred Education and Research Foundation (TERF). Both organizations share a commitment to enhancing the welfare and long-term success of Thoroughbreds beyond the racetrack. Shifting Strides not only focuses on individualized retraining and responsible adoption, but also emphasizes the importance of education and scientific research to improve outcomes for transitioning racehorses. This mission complements TERF’s “Goal B” focus on supporting initiatives that promote equine research and evidence-based advancements in Thoroughbred care. By integrating hands-on retraining with research into hoof health, biomechanics, and post-racing adaptation, Shifting Strides embodies TERF’s vision of leveraging knowledge and innovation to create a more sustainable and humane future for Thoroughbreds.

--- 2. Briefly outline 3-5 goals for the requested funds and how these goals support your mission. ---

The equine hoof is a biologically complex structure essential for weight-bearing and locomotion, especially in high-performance racehorses. However, the molecular composition of healthy hoof tissue at the point of retirement—when horses are transitioning to new careers—remains poorly characterized. Anecdotally, many owners of off-the-track racehorses note hoof problems in their ex-racehorses, including extensive hoof cracking, soreness, and bruising. It therefore stands to reason that there is additional knowledge to be gained regarding how the racehorse hoof changes during early retirement from racing, with a particular emphasis on how proteins intrinsic to the hoof structure are altered once the racing career has ended.

Goal 1: Establish the molecular baseline of hoof health in recently retired racehorses We will use high-resolution mass spectrometry-based proteomics and metabolomics to deeply profile the structural proteins in hoof wall and sole tissue. From this we will obtain the deepest profiling of the racehorse hoof to date and capture the hoof at a molecular level at the end of the racing career. This will establish a baseline for understanding how the hoof further changes as the horse progresses to a second career and retirement from the racetrack.

Goal 2: Define how hoof tissue remodels during the early post-racing transition Many racehorses face hoof-related challenges during the transition to second careers due to shifts in trimming practices, biomechanical demands, and reduced farrier oversight. Using a longitudinal approach, we will reveal dynamic remodeling processes, stress-response mechanisms, and potential failure points in tissue adaptation as horses transition careers.

Goal 3: Translate molecular findings into actionable insights for hoof care Current assessments of hoof health rely on subjective evaluation, lacking molecular-level indicators that could guide preventive care. By combining static and longitudinal molecular data from hoof tissues, we aim to identify biomarkers and mechanistic pathways that can inform targeted trimming strategies, early interventions, and nutritional or therapeutic approaches. These findings will provide farriers, veterinarians, and caretakers with objective molecular tools to improve the hoof integrity and long-term soundness of off-track racehorses entering new careers.

Goal 4: Disseminate results to the equestrian community and provide a comprehensive dataset of the hoof We anticipate that the mass spectrometry dataset generated by this project will be one of the largest proteomic profiling of the equine hoof to date. We anticipate identifying hundreds of proteins within the hoof, both from the hoof itself and from microbes and bacteria present. We will prepare our results for publication for both a scientific audience (by publishing in a scientific journal) and a general equestrian audience (by publishing in Equus or similar publication). We will also deposit our raw data to a proteomics repository so that other equine researchers can analyze and independently benefit from the results.

These goals are in line with our non-profit mission to provide successful second careers to racehorses and to further research and education for thoroughbreds as they transition from the racetrack.

--- 3. Provide a detailed description of the proposed project, how it is related to the mission of TERF and how it will impact the health and welfare of the horse. (Note: research applications should be understandable to a non-scientific audience and include sufficient detail and rigor for the scientific reviewers.) ---

The equine hoof is a highly specialized and biologically complex structure essential for locomotion, shock absorption, and overall performance in horses. Nowhere is optimal hoof function more critical than in the racing industry, where peak athletic performance and long-term soundness are paramount. Despite the hoof’s vital role, the molecular underpinnings of hoof integrity, disease susceptibility, and response to trimming or shoeing remain poorly understood. Disorders such as laminitis, white line disease, and hoof wall separation contribute significantly to lameness, early retirement, and economic loss in racehorses. Currently, hoof health is assessed through subjective evaluations and limited histopathological analysis, underscoring a pressing need for molecular-level insights.

The transition from racing to a second career represents a pivotal period in a racehorse’s life, often marked by substantial changes in hoof loading, trimming regimes, and physical demands. Despite their athletic conditioning, many retiring racehorses experience hoof-related challenges that limit their suitability for new roles in sport, therapy, or leisure. Poor hoof conformation, structural imbalances, and subclinical damage acquired during training and racing can manifest as lameness or chronic hoof disorders once the horse exits the high-maintenance environment of the track. These issues not only compromise welfare but also reduce the success rate of rehoming and long-term soundness in off-track Thoroughbreds Mass spectrometry (MS)-based approaches, including proteomics, metabolomics, and lipidomics, offer powerful tools to interrogate the molecular landscape of hoof tissues and systemic biomarkers of hoof health. By applying these technologies to horses at the point of retirement and during retraining, we can define molecular signatures associated with hoof strength, remodeling, and inflammatory stress. This knowledge will enable early detection of hoof compromise, guide personalized trimming or supplementation strategies, and ultimately improve the success and longevity of second careers for these valuable animals.

The project will be comprised of three aims:

Aim 1: Characterize the molecular composition of hoof tissue in racehorses at the time of retirement using mass spectrometry-based proteomics and metabolomics.

We will collect hoof wall and sole tissue from recently retired racehorses and analyze them using high-resolution LC-MS/MS. Collection of hoof samples will be done in collaboration with our farrier, Peter Schioppo (based in Southbury, CT). We will quantify structural proteins (e.g., keratins, desmosomal components), crosslinking enzymes, and lipid species (e.g., ceramides, cholesterol derivatives) to define the baseline molecular landscape of the hoof capsule in this population. We will aim to produce a deep profiling of proteins in the equine hoof immediately after conclusion of racing career that has not previously achieved by using state-of-the-art mass spectrometry instrumentation. We will perform deep mass spectrometry protein sequencing to obtain the most comprehensive proteome possible, with the goal of identifying the most proteins in the racehorse hoof to date.

Aim 2: Identify longitudinal changes in the hoof tissue proteome during the early post-racing transition period.

As racehorses adapt to new mechanical demands and trimming routines in their second careers, their hooves undergo structural and biochemical remodeling. However, the temporal dynamics of this remodeling at the molecular level remain unknown. In this aim, we will collect hoof wall and sole tissue samples (e.g., trimmings) from a cohort of recently retired racehorses at multiple time points over a 6-month period. Using quantitative LC-MS/MS proteomic analysis, we will track changes in key structural proteins, extracellular matrix components, and stress-response proteins. To best capture changes in the hoof protein amounts over time in a highly rigorous way, we will use tandem-mass tag (TMT) quantitative labeling where samples are labeled with chemical tags to facilitate simultaneous analysis of different samples and therefore measuring relative protein amounts across samples. By integrating these data with farrier evaluations and hoof conformation measurements, we aim to define time-dependent molecular pathways associated with either adaptation or deterioration. These insights will help establish molecular benchmarks for healthy hoof remodeling and identify windows for intervention during the transition out of racing.

Aim 3: Define the molecular response of hoof tissue to microbial infection in cases of thrush and other hoof-associated bacterial or fungal diseases.

Infectious conditions such as thrush and white line disease are common in horses, particularly during periods of environmental transition and decreased hoof maintenance, such as after retirement from racing. These infections can lead to tissue degradation, lameness, and chronic hoof instability, yet the host’s molecular response to microbial invasion remains largely uncharacterized. In this aim, we will use untargeted proteomics and metabolomics to compare hoof tissue samples from affected and unaffected horses. Tissue will be collected from the frog and surrounding regions during routine farrier care or veterinary treatment. We will identify changes in host proteins involved in inflammation, matrix degradation, and innate immune responses, as well as metabolic shifts associated with tissue breakdown and microbial activity. Where feasible, microbial peptides or metabolites will also be characterized to gain insight into pathogen composition and virulence factors. This study will reveal the biochemical signatures of infection and tissue compromise, offering potential biomarkers for early detection and guiding targeted treatment strategies.

--- 4. Provide a timeline detailing the expected progress of the project and specific milestones. ---

We propose two years to complete this project. The first year and a half will be dedicated to gathering hoof samples from horses as they transition from the racetrack. We will gather samples from at least 8 horses and take hoof trimming samples every month for 6 months, starting from their first trim off the track. As samples are gathered they will be prepared for mass spectrometry and analyzed. The second year will be dedicated to finishing the sample collection, finishing mass spectrometry analysis, and preparing the results for publication in both a scientific journal focused on equine health, as well as an article prepared for the general public (to be published in a magazine such as Equus). At the conclusion of the study we will make the dataset publicly available via a mass spectrometry data repository.

We anticipate that this study will be of interest to numerous other researchers as well, as it will be one of the largest proteomic level analysis of the hoof to date. Previous studies conducted over 10 years ago identified a host of proteins in the equine hoof, however mass spectrometry has seen technology advance in leaps and bounds, which has facilitated more comprehensive identification of low-abundance proteins and deeper sample coverage. We fully anticipate that we will be able to comprehensively profile and characterize hundreds of proteins in the equine hoof, and produce one of the most comprehensive molecular level atlases of the hoof to date.

Timeline and Milestones:

Months 0-6. Collect samples from first cohort of least three horses. Monthly sample collections will occur during regular trimming and shoeing appointments (scheduled for every 4 weeks). As samples are collected they will be prepared for mass spectrometry to limit sample degradation during storage.

Milestone: samples are collected and continual progress is made towards including more horses in the study. First samples are run on the mass spectrometer by the end of the first 6 months.

Months 6-12. Collect samples from 4-6 horses. By the end of month 12 the samples from cohort 1 are analyzed on the mass spectrometer.

Milestone: Completion of the sample collection

Months 12-18. All remaining samples will be run on the mass spectrometer. Data analysis will continue. Figures will be prepared

Milestone: Completion of sample analysis

Months 18-24: Data will be analyzed and analysis will be completed. Manuscripts for publications will be prepared and raw data will be uploaded to repositories.

Milestone: Dissemination of data and findings

--- 5. Provide a detailed budget for the projected use of the funds. (Note: no funds will be provided for administrative overhead or capital spending; TERF reserves the right to modify funding based on Foundation requirements). Attach budget to submitted proposal as needed. ---

We are requesting $15,000 to cover the cost of the project.

The majority of these funds will be dedicated to paying for instrument run-time for mass spectrometry. We propose to use the state-of-the-art mass spectrometers at the University of Connecticut. Instrument rates are as follows: A 1 hour run on a high-resolution ThermoFisher Eclipse Mass Spectrometer is $122. We anticipate having 12 samples per horse (6 time points for trimming, front and hind hoof sample) from 6 horses, for a total of 72 total samples to be analyzed by mass spectrometry. This would then be an anticipated total of $8784 for Aim 1 and Aim 2. In addition, we anticipate an additional $732 for mass spectrometry analysis of the frog in Aim 3. Typically a sample-preparation charge is also necessary for mass spectrometry, however our co-founder Dr. Battison is an expert in mass spectrometry and is capable of performing the sample prep, saving these charges. For Aim 2 we will need to purchase TMT labeling reagents which cost $603 for a 6-plex kit, which is sufficient for analysis of one horse over 6 months of sample collection. The total for TMT reagents will therefore be approximately $4000 total for n= 6 horses. The only remaining budgetary needs will be to pay for chemical reagents (such as acetonitrile, ammonium bicarbonate, high purity water, lysis buffers), proteases (such as trypsin), and consumables (clean-up columns, tubes), which we anticipate will cost around $2000-$2500 for completing all 3 aims of the study.

--- 6. Provide a list of all other sources of funding and the amount(s) received. ---

no other funding has been received as of the submitting date for this grant

--- 7. Briefly summarize your charity's past public education and research efforts. ---

We are a new non-profit and have been focused on establishing our facility. In April 2024 we attended the University of Connecticut Equine Extension clinic day where we had a booth where we provided information on thoroughbred alternative careers and successful strategies for re-starting of OTTBs. We plan to attend Equine Affair in 2026 with a similar booth, and we plan on creating documents and web-resources for people to educate on proper thorougbred nutrition, hoof health, and training practices that will be provided freely online and provided whenever someone adopts one of our horses.

--- 8. If you received funding from TERF previously, describe how these funds were used and outcomes achieved. Include any relevant publicity your charity received relating to the funding. (i.e.: media coverage, such as news articles, scientific publications, provide links to copies, as appropriate). ---

no funding from TERF has been received previously

--- 9. List other organizations or major contributors that have provided funding to your organization in the last calendar/fiscal year. For research grant applications, provide a list of all current funding relating to your current proposal. ---

no major funding or contributors at this time, as we were founded in 2024

--- 10. Name a responsible person with whom TERF may communicate regarding specific questions and who will be responsible for follow-up information regarding the project. ---

Alexandria Battison

--- 11. Provide appropriate references to support the proposed research. ---

1. Kiatthitinan, P., Yee, D. H., Tran, H. M. Q. & Sun, B. Advantages of Incomplete Digestion in Human Hair Shaft Proteomics, a Focus on Cuticular Keratins. Rapid Commun Mass Spectrom 39, e10071 (2025).

2. Blanco-Pintos, T. et al. Characterisation of the periodontal proteome in gingival crevicular fluid and saliva using SWATH-MS. Front Cell Infect Microbiol 15, 1576906 (2025).

3. Blanco-Pintos, T. et al. Characterisation of the periodontal proteome in gingival crevicular fluid and saliva using SWATH-MS. Front Cell Infect Microbiol 15, 1576906 (2025).

4. Wertz, P. W. & Downing, D. T. Cholesteryl sulfate: the major polar lipid of horse hoof. J Lipid Res 25, 1320–1323 (1984).

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6. Dong, S.-W. et al. Comparative proteomics analysis provide novel insight into laminitis in Chinese Holstein cows. BMC Vet Res 11, 161 (2015).

7. Szabó, L., Pollard, D. & Nagy, A. Computed tomographic measurements in 110 front hooves of non-lame Thoroughbred racehorses and Warmblood showjumpers. Equine Vet J (2025) doi:10.1111/evj.14509.

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10. Petrov, K. K. & Dicks, L. M. T. Fusobacterium necrophorum, and not Dichelobacter nodosus, is associated with equine hoof thrush. Vet Microbiol 161, 350–352 (2013).

11. Identification of rhinoceros keratin using direct analysis in real time time‐of‐flight mass spectrometry and multivariate statistical analysis - Price - 2018 - Rapid Communications in Mass Spectrometry - Wiley Online Library. <https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/rcm.8285>.

12. Horan, K. et al. Influence of Speed, Ground Surface and Shoeing Condition on Hoof Breakover Duration in Galloping Thoroughbred Racehorses. Animals (Basel) 11, 2588 (2021).

13. Carter, R. A., Shekk, V., de Laat, M. A., Pollitt, C. C. & Galantino-Homer, H. L. Novel keratins identified by quantitative proteomic analysis as the major cytoskeletal proteins of equine (Equus caballus) hoof lamellar tissue1. Journal of Animal Science 88, 3843–3855 (2010).

14. Fürst, A. E. & Lischer, C. J. Other Clinical Problems of the Equine Foot. Vet Clin North Am Equine Pract 37, 695–721 (2021).

15. Annan, R. et al. Racehorse welfare across a training season. Front Vet Sci 10, 1208744 (2023).

16. Kuwano, A., Ueno, T., Katsurashima, Y., Tateno, O. & Saitoh, S. Selenium deposition in an atypically disintegrated hoof wall in a Thoroughbred racehorse with alkali disease: Proof by energy-dispersive X-ray fluorescence analysis. J Comp Pathol 212, 51–55 (2024).

17. Horan, K., Price, H., Day, P., Mackechnie-Guire, R. & Pfau, T. Timing Differences in Stride Cycle Phases in Retired Racehorses Ridden in Rising and Two-Point Seat Positions at Trot on Turf, Artificial and Tarmac Surfaces. Animals (Basel) 13, 2563 (2023).

--- 12. How many Executive Staff and Board of Directors does your organization have? ---

2

--- Director Name (1) ---

Alexis Bradley

--- Director Address (1) ---

161 Hubbard Street

Middlefield, CT

06455

US

--- Director Work Phone (1) ---

+18603384153

--- Director Home Phone (1) ---

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--- Director Name (2) ---

Alexandria Battison

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--- Director Work Phone (2) ---

+18184547915

--- Director Home Phone (2) ---

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--- 1. Name - Job Title ---

Alexis Bradley - Director, Barn Manager

--- 1. Salary ---

0

--- 1. Duties ---

Feeding and turnout, daily management of horses, primary trainer for horses being re-started,

--- 2. Name - Job Title ---

Alexandria Battison - Director,

--- 2. Salary ---

0

--- 2. Duties ---

Responsible for educational efforts, fundraising, and grants, as well as also training and re-starting horses

--- 3. Name - Job Title ---

Sienna Connolley - volunteer

--- 3. Salary ---

0

--- 3. Duties ---

turnout horses 3 mornings a week and make feeds and clean stalls on those days