



# List of Projects Funded by the Foundation

## 2021 – 2025

-  **Projects Supported: 34**
-  **Funding Commitment: HKD 42.5 million**
-  Click Project Titles for details of the award

### 20 Active Projects

#### Degenerative orthopaedic disease and fractures

- ▶ **Addressing senescence-associated tendon degeneration using small molecule inhibitors for effective injury prevention.**  
Principal Investigator: Roger Smith  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 300,657 - Major Research Grant 2024  
Project Period: January 2025 - January 2028
- ▶ **Characterising the epigenetic landscape of mesenchymal stem cells in relation to tendinopathies and aging**  
Principal Investigator: Androniki Psifidi  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 30,845 - Pump-prime Funding 2024  
Project Period: March 2025 - February 2026
- ▶ **Development of a CT-based biomarker model of fetlock joint disease**  
Principal Investigator: Chris Kawcak  
Administering Institution: Colorado State University, US  
Amount Awarded: USD 314,914 - Major Research Grant 2023  
Project Period: November 2023 - October 2026
- ▶ **Investigation of equine fetlock joint immunopathology and the immunomodulatory effects of intra-articular therapeutics**  
Principal Investigator: Heidi Reesink  
Administering Institution: Cornell University, US  
Amount Awarded: USD 186,055 - Research Training Scholarship 2021  
Project Period: January 2022 - December 2025
- ▶ **Mathematical modelling of metacarpal subchondral bone adaptation, microdamage, and repair in racehorses**  
Principal Investigator: Peta Hitchens  
Administering Institution: The University of Melbourne, Australia  
Amount Awarded: AUD 369,702 - Major Research Grant 2023  
Project Period: March 2024 - March 2026
- ▶ **Mesenchymal stem cell licensing for improved tendon healing**  
Principal Investigator: Lauren Schnabel  
Administering Institution: North Carolina State University, US  
Amount Awarded: USD 164,640 - Major Research Grant 2022  
Project Period: June 2023 - May 2025
- ▶ **Multiscale modeling of subchondral bone fatigue injury at the equine fetlock joint**  
Principal Investigator: W. Brent Edwards  
Administering Institution: University of Calgary, Canada  
Amount Awarded: CAD 136,042 - Research Training Scholarship 2023  
Project Period: May 2024 - April 2028

## Degenerative orthopaedic disease and fractures (*Continued*)

### ▶ Prediction of limb injury using inertial sensor data from Thoroughbred racehorses training and racing

Principal Investigator: Chris Whitton  
Administering Institution: The University of Melbourne, Australia  
Amount Awarded: AUD 570,226 - Major Research Grant 2024  
Project Period: February 2025 - February 2028

### ▶ Risk assessment for condylar stress fracture in the racing thoroughbred

Principal Investigator: Peter Muir  
Administering Institution: University of Wisconsin-Madison, US  
Amount Awarded: USD 338,278 - Major Research Grant 2022  
Project Period: April 2023 - July 2026

### ▶ Use of mRNA expression and serum biomarkers as tools to monitor musculoskeletal adaptation in Thoroughbred horses returning to race training

Principal Investigator: Allen Page  
Administering Institution: University of Kentucky, US  
Amount Awarded: USD 379,495 - Major Research Grant 2023  
Project Period: July 2024 - December 2026

## Diseases associated with intensive training

### ▶ Applying novel multi-omic approaches to investigate the impact of training on airway immunity and molecular pathways underpinning MMEA and EIPH

Principal Investigator: Scott Pirie  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 181,243 - Major Research Grant 2021  
Project Period: August 2022 - July 2025

### ▶ Defining a transcriptomic signature for equine recurrent laryngeal neuropathy

Principal Investigator: Richard Piercy  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 189,144 - Research Training Scholarship 2021  
Project Period: April 2022 - October 2025

### ▶ Does Vitamin D deficiency have a role to play in Recurrent Exertional Rhabdomyolysis?

Principal Investigator: Charlotte Maile  
Administering Institution: University of Surrey, UK  
Amount Awarded: GBP 12,040 - Pump-prime Funding 2024  
Project Period: December 2024 - November 2025

### ▶ Interdependence of structure and function: using electroanatomic mapping and MRI to determine the tissue characteristics driving propagation in the equine heart

Principal Investigator: John Keen  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 30,732 - Pump-prime Funding 2024  
Project Period: December 2024 - November 2025

### ▶ Mapping the equine cardiac channelome – elucidation of molecular targets of electrophysiological function in horses with and without cardiac rhythm abnormalities.

Principal Investigator: Rebecca Lewis  
Administering Institution: University of Surrey, UK  
Amount Awarded: GBP 179,036 - Major Research Grant 2021  
Project Period: October 2022 - December 2025

## Diseases associated with intensive training (*Continued*)

### ► Unravelling the toxic and pro-inflammatory potential of inhalable mineral dusts generated from racehorse working surfaces

Principal Investigator: Michela Bullone  
Administering Institution: University of Turin, Italy  
Amount Awarded: EUR 32,500 - Pump-prime Funding 2023  
Project Period: April 2024 - April 2025

## Husbandry and management

### ► Implementation of Qualitative Behaviour Assessment as a tool to improve racehorse welfare/Quality of Life (QoL)

Principal Investigator: Gemma Pearson  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 207,312 - Major Research Grant 2024  
Project Period: June 2025 - June 2028

### ► Looking on the brighter side of life – Characterising the expression of positive emotion in Thoroughbred horses

Principal Investigator: Hayley Randle  
Administering Institution: Charles Sturt University, Australia  
Amount Awarded: AUD 325,693 - Major Research Grant 2022  
Project Period: January 2023 - January 2026

## The biological integrity of the horse

### ► MULTITRACE MS - MULTIpLexed TRAnsgene deteCtion in the Equine by Mass Spectrometry

Principal Investigator: Mario Thevis  
Administering Institution: German Sport University Cologne, Germany  
Amount Awarded: EUR 327,735 - Major Research Grant 2024  
Project Period: February 2025 - February 2028

## Others

### ► Host immune response against EHV-1: a novel approach.

Principal Investigator: Lutz Goehring  
Administering Institution: University of Kentucky, US  
Amount Awarded: USD 344,647 - Major Research Grant 2024  
Project Period: March 2025 - November 2027

## 14 Completed Projects

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### Degenerative orthopaedic disease and fractures

### ► Effect of surface and predominant direction of training and racing on movement symmetry and hoof shape in racing Thoroughbreds

Principal Investigator: Thilo Pfau  
Administering Institution: University of Calgary, Canada  
Amount Awarded: CAD 236,303 - Major Research Grant 2021  
Project Period: September 2022 - August 2024

### ► Immunomodulatory effects of equine chondroprogenitor cells in an animal model of osteoarthritis

Principal Investigator: Laurie Goodrich  
Administering Institution: Colorado State University, US  
Amount Awarded: USD 37,935 - Pump-prime Funding 2021  
Project Period: April 2022 - September 2023

## Degenerative orthopaedic disease and fractures (*Continued*)

- ▶ **Real-time risk prediction for thoroughbred racing at The Hong Kong Jockey Club**  
Principal Investigator: Tim Parkin  
Administering Institution: University of Bristol, UK  
Amount Awarded: GBP 30,353 - Major Research Grant 2021  
Project Period: January 2022 - July 2023
  
- ▶ **Relationship between Thoroughbred racing and training workloads and the fatigue life of equine subchondral bone**  
Principal Investigator: Chris Whitton  
Administering Institution: The University of Melbourne, Australia  
Amount Awarded: AUD 243,404 - Major Research Grant 2021  
Project Period: April 2022 - September 2024
  
- ▶ **Unravelling the genetic mechanisms underlying fracture risk in horses**  
Principal Investigator: Debbie Guest  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 164,850 - Major Research Grant 2021  
Project Period: November 2022 - October 2024

## Diseases associated with intensive training

- ▶ **Investigation of cell free DNA as a biomarker of NETosis in airway lavage samples from horses with neutrophilic asthma**  
Principal Investigator: Mary Sheats  
Administering Institution: North Carolina State University, US  
Amount Awarded: USD 38,507 - Pump-prime Funding 2022  
Project Period: July 2023 - December 2024
  
- ▶ **Prediction and prophylaxis of EIPH during racing by on-board monitoring of horses during training – a pilot study**  
Lead Applicant: Emmanuelle Van Erck-Westergren  
Host Organisation: Equine Sports Medicine Practice, Belgium  
Amount Awarded: EUR 13,488 - Small Research Project 2021  
Project Period: February 2022 - January 2023
  
- ▶ **The effects of short-term omeprazole on serum gastrin and chromogranin A, as markers of rebound gastric hyperacidity, in the horse.**  
Principal Investigator: Benjamin Sykes  
Administering Institution: Massey University, New Zealand  
Amount Awarded: NZD 33,235 - Pump-prime Funding 2021  
Project Period: January 2022 - December 2022

## Husbandry and management

- ▶ **Automatic behaviour recognition using wearable sensors for improving horse health and welfare**  
Principal Investigator: Kai Liu  
Administering Institution: City University of Hong Kong, HKSAR, China  
Amount Awarded: HKD 294,987 - Pump-prime Funding 2022  
Project Period: June 2023 - May 2024

## Husbandry and management (*Continued*)

- ▶ **Bugs, Bones and Vitamin D - A pilot study: Developing novel tools to assess bone health and reduce the risk of bone fractures.**

Principal Investigator: Chris Proudman  
Administering Institution: University of Surrey, UK  
Amount Awarded: GBP 19,223 - Pump-prime Funding 2023  
Project Period: November 2023 - October 2024

- ▶ **Developing novel tools towards the reduction of antibiotic usage and against antimicrobial resistance in equine infection, by using stem cell approaches**

Principal Investigator: Cristina Esteves  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 30,261 - Pump-prime Funding 2023  
Project Period: April 2024 - January 2025

- ▶ **The development and validation of novel behavioural assessment methods for equine welfare**

Principal Investigator: Catherine Dwyer  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 24,627 - Pump-prime Funding 2021  
Project Period: February 2022 - February 2023

## Potential for racehorses to adapt to different careers on retirement from racing

- ▶ **Transforming retraining: using multidisciplinary expert consensus to improve success rates in racehorses' second careers**

Principal Investigator: Jane Williams  
Administering Institution: Hartpury University, UK  
Amount Awarded: GBP 27,830 - Pump-prime Funding 2022  
Project Period: January 2023 - June 2024

## Others

- ▶ **Equine influenza virus epigenetically imprints airway basal cells and alters chronically the airway epithelium repair potential.**

Principal Investigator: Caroline Chauche  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 27,582 - Pump-prime Funding 2021  
Project Period: February 2022 - January 2023

## Project Summary from Research Team (by field of research and project title)

### Degenerative orthopaedic disease and fractures

#### Addressing senescence-associated tendon degeneration using small molecule inhibitors for effective injury prevention.

Principal Investigator: Roger Smith  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 300,657 - Major Research Grant 2024  
Project Period: January 2025 - January 2028  
Grant Reference No.: MRG-241027  
Status: [Active](#)  
Field of Research: Musculoskeletal injury and disease / Basic science of musculoskeletal system / Strategies to prevent musculoskeletal disease and injury / Orthopaedic disease / Molecular biology / Genetics / Inflammation

*Brief Summary: The project primarily focuses on two of the key topics in the objectives of the Foundation - reducing the incidence of disease and injury in racehorses and developing management practices that improve the health and welfare of racehorses, while training, racing and in retirement.*

Tendon injury is a major cause of 'wastage' in the racehorse. Although sudden in onset, these injuries are believed to be preceded by age-related degeneration and followed by dysfunctional healing. Clearly, the ability to prevent the initial process of degeneration and/or enhance repair post-injury would provide an effective strategy for both prevention and treatment of this common condition. The mechanism of degeneration is not well understood but the molecular changes identified are hallmarks of new mechanisms related to how cells cope with stress and have been implicated in a number of age-related degenerative diseases in animal models and humans. This unique collaboration between three universities provides the tools to investigate the relative importance of each mechanism in the laboratory in live tendon subjected to relevant stresses. We can also then test novel candidate molecules that can slow or stop the degenerative process, which, because they are small molecules, could subsequently be delivered transdermally in live horses.

### Characterising the epigenetic landscape of mesenchymal stem cells in relation to tendinopathies and aging

Principal Investigator: Androniki Psifidi  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 30,845 - Pump-prime Funding 2024  
Project Period: March 2025 - February 2026  
Grant Reference No.: PPF-242023  
Status: [Active](#)  
Field of Research: Musculoskeletal injury and disease / Improved treatment of musculoskeletal disease and injury / Molecular biology / Genetics

*Brief Summary: We aim to characterise MSC alterations with disease and aging to improve future therapeutics of tendinopathies and initiate a database of samples useful for future functional genomic studies aiming to identify biomarkers with predictive capacity for tendinopathies. These objectives align well with the core values and objectives of the Foundation.*

Equine athletes are under a tremendous amount of stress in their limbs which often leads to various lameness issues. One of the most common injuries are tendon injuries (tendinopathies) and one of the hardest to recover from. The disease is difficult to manage, compromises welfare and performance, has often a poor prognosis, and is a major frustration for owners and vets. One of the most promising therapeutic strategies that enhance regeneration of the injured tendon are cell-based therapies. Although we know that cell-based therapies (mesenchymal stem/stromal cells (MSCs) therapies) have beneficial effects on tendon healing, we do not know the mechanisms driving this improved healing process. Here, we will use advanced sequencing technologies to characterise MSCs from healthy horses and horses with tendinopathies as well as from aging horses. This can provide a foundation for improving the efficacy of cell therapies as well as increase our understanding of the impact of disease and aging in these therapies. In horses, the disorder has a genetic

basis but specific causes have not been identified so far. Here, we will collect residual samples from horses with tendinopathies and create a database to be used in future studies aiming to develop novel prognostic tests for identifying at-risk animals and drug targets for novel treatments.

### **Development of a CT-based biomarker model of fetlock joint disease**

Principal Investigator: Chris Kawcak  
Administering Institution: Colorado State University, US  
Amount Awarded: USD 314,914 - Major Research Grant 2023  
Project Period: November 2023 - October 2026  
Grant Reference No.: MRG-2023-231031  
Status: **Active**  
Field of Research: Musculoskeletal injury and disease / Basic science of musculoskeletal system / Early diagnosis of musculoskeletal disease and injury / Imaging / Orthopaedic disease

*Brief Summary: The objectives of the project are to improve the diagnostic and predictive characterization of musculoskeletal injuries in racehorses using computed tomography. This aligns with the Foundation's objectives of reducing injury and informing management practices to optimize the health and welfare of racehorses.*

Catastrophic injury in the thoroughbred racehorse is unfortunately common, and the industry's social license to continue horse racing has come under scrutiny in large part because of severe injuries to the fetlock joints. The scientific community has worked to describe the microstructural changes that occur in the joints of injured horses, and advances in diagnostic imaging (namely to development of computed tomography - CT) have allowed veterinarians to identify horses that might be at risk of injury when a small fracture is present. However, identification of the microstructural changes that occur in the subchondral bone of racehorses prior to fracture formation would be ideal and allow the veterinary community to identify horses at risk of injury sooner in their career to better monitor and manage their training. Based on this premise, the investigators plan to develop analysis techniques from CT-based data that can be used to characterize the microstructural changes that commonly occur with fetlock joint injury. Specifically, the investigators aim to use well-established shape determination and textural correlation techniques from CT data to discriminate diseased from normal bone and then apply machine learning techniques to those data to develop a software model that can be used for the clinical determination of fracture risk.

Specifically, we hypothesize that joint shape, subchondral bone architecture, bone lysis and sclerosis patterns, as represented by image-based shape and texture features, will interdependently predict susceptibility of the bones to injury. We will use two specific aims to address this hypothesis. Specific Aim 1: To develop a reference morphologic model of the equine fetlock joint using UHR CT in normal and abnormal joints of racehorses. Based on strong microCT evidence in the literature, we hypothesize that there will be a difference in model characteristics between the 2 populations of horses, allowing for optimization in recognition of those differences. Specific Aim 2: To correlate model characteristics between the findings of Specific Aim 1 and results from other fan- and cone-beam systems currently available for equine imaging. We hypothesize that shape and texture findings on current clinical scanners can be correlated back to the results of the reference UHR CT. The results of this study will help to inform clinicians as to characteristics that may predispose horses to injury versus those that are a response to intense modeling and might actually be protective to the horse. These results can form the base model onto which variables external to the horse can be overlaid.

### **Effect of surface and predominant direction of training and racing on movement symmetry and hoof shape in racing Thoroughbreds**

Principal Investigator: Thilo Pfau  
Administering Institution: University of Calgary, Canada  
Amount Awarded: CAD 236,303 - Major Research Grant 2021  
Project Period: September 2022 - August 2024  
Grant Reference No.: MRG-2021-101379  
Status: **Completed**  
Field of Research: Locomotor biomechanics / Performance and injury / Equine behaviour / Animal welfare / Wearable device

#### Publication and Presentation:

- ▶ Forbes, Bronte, Winnie Ho, Rebecca S. V. Parkes, Maria Fernanda Sepulveda Caviedes, Thilo Pfau, and Daniel R. Martel. 2024. "Associations between Racing Thoroughbred Movement Asymmetries and Racing and Training Direction" *Animals* 14, no. 7: 1086. <https://doi.org/10.3390/ani14071086>
- ▶ Chan, Z. (2024, Oct). Association between Hoof and Movement Asymmetry In Racing Thoroughbreds by Dr. Zoe Chan. Youtube. <https://youtu.be/YVyyLjTC1ps?si=vLe-hW7src1wKgZb>

### Plain Language Summary

This project has investigated how training direction, hoof shape, and surface type affect the movement of Thoroughbred racehorses, with the aim of improving clinical assessments 'lameness and poor performance' exams and in turn reducing injury risk by providing improved evidence-based decision criteria for 'catching developing problems' early on. Our findings showed that horses trained predominantly in one direction - either clockwise or counterclockwise as typically done in different countries - show movement asymmetries, related to how the horses use their forelimbs and hindlimbs to adjust force distribution when asked to consistently perform on racecourses with clockwise or anticlockwise curves.

Horses showed wider hooves on the legs that are on the inside of the turn. Anecdotally, wider hooves are seen as a sign of increased force production, since horn growth reacts to loading patterns. Our findings are contradicting previous measurements in trotting horses, indicating increased force production with the limb on the outside of the circle. This suggests different loading mechanisms across quadrupedal gaits. We suggest to further investigate this.

Horses with difference in hoof width between left and right limbs showed movement patterns indicative of increased loading of the limb on the side of the wider hoof when being asked to trot in straight lines. This suggests that differences in hoof shapes between left and right limbs are an important factor for equine lameness examinations, where movement symmetry are important lameness indicators.

Finally, we examined how different surfaces, with particular emphasis on relevance for Thoroughbred racehorses (turf and sand-based) and movement directions (straight vs. circular lungeing), influence movement asymmetry. Horses showed different patterns of movement on turf and sand, with more pronounced asymmetries observed for the head on turf and for the withers on sand. This is interesting for untangling how horses shift force between front and hind limbs, since the relationship between head and withers movement has been found to help locate the primary source of a lameness (front versus hind limb).

Circular lungeing generally exaggerated movement imbalances, with subtle differences in specific directions, i.e. either in the same direction or opposite to a horse's normal racing direction. These specific adaptations, observed on specific surfaces should be used to inform clinical decision-making during lameness examinations in Thoroughbred racehorses.

Overall, this research project has provided fundamental insights into how the predominant direction of performance is related to hoof shape and movement symmetry. Both are important for lameness examinations in horses. Catching a developing problem early can in turn lead to earlier diagnostics and implementation of remedial action (veterinary intervention and/or adaptation of training regimen) and contribute to improved racehorse welfare and performance. Drawing attention to hoof shape and specific movement patterns during circular movement on Thoroughbred-specific surfaces will contribute to improved recommendations for lameness examinations.

### Key Findings and Outcomes of the Project

**Movement Asymmetry in Relation to Directional Training (WP1):** WP1 analysed movement asymmetry in 522 Thoroughbred racehorses and revealed adaptations to predominant directional training. Data was collected from horses trained in clockwise direction and horses trained in an anticlockwise direction. Using two inertial measurement units (IMUs, poll, sacrum), vertical head and pelvic displacement was obtained from the two halves of a stride cycle. Seven variables of interests were obtained, and chi-squared tests conducted to identify differences in the number of horses with left- or right-sided movement asymmetry between the two cohorts.

Compared against the cohort that trained predominantly anticlockwise, the clockwise cohort had significantly more horses with left forelimb asymmetry ( $p < 0.03$ ). When compared within the anticlockwise cohort, there were more horses with right fore- ( $p < 0.01$ ) and hindlimb ( $p < 0.02$ ) asymmetrical than left. The findings suggest a compensatory mechanism, since direct force measurement (in trot) have suggested increased force production with the outside (fore-)limb. Hence, the horses in our study appear to adjust force distribution based on training direction. Further research is needed to explore the underlying physiological and biomechanical processes and to assess the long-term effects of directional training on musculoskeletal health and performance in racehorses.

**Hoof Shape and Movement Asymmetry (WP2):** WP2 analysed hoof shape asymmetries in Thoroughbred racehorses training/racing in a clockwise direction. Distal limb photographs of all four hooves, capturing the solear aspect, were used to assess hoof width at the end of a shoeing cycle. Hoof width was determined at the widest part of the hoof perpendicular to the midline (Figure 1). Paired t-tests compared the inside (right) and outside (left) hoof widths for both fore- and hindlimbs. Our analysis revealed significant differences between the width of the inside and outside limbs (Figure 1), with horses exhibiting wider inside hooves. On average, inside hooves were 2.4 mm and 2.1 mm wider in the fore- and hindlimbs, respectively.

Using the same IMU-based system as in WP1, a sub-group analysis was conducted between horses with wider inside and wider outside fore- and hindlimbs using independent-samples t-tests. Variables of interest included within-stride differences in total range of vertical movement (UpDiff), minimum (MinDiff) and maximum (MaxDiff) vertical displacement. Significant differences were found for head and withers for UpDiff and MaxDiff (Figure 2), but not for pelvic movement ( $p > 0.05$ ).

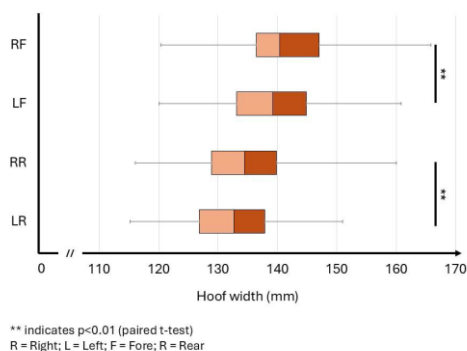


Figure 1. Hoof width (mm), results of paired t-tests (left). Illustration of hoof width measurement (right).

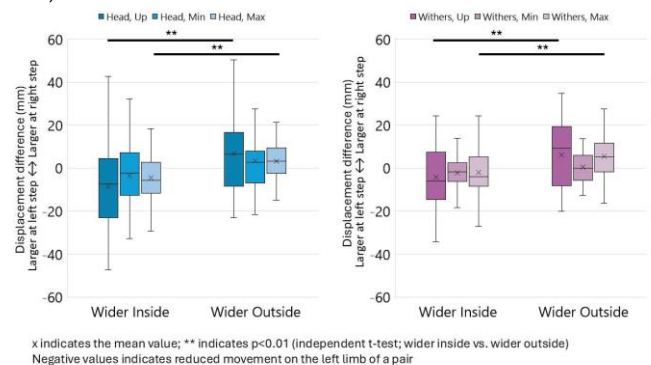


Figure 2. Boxplots of within-stride vertical displacement difference (mm) for head (left) and withers (right). Results of independent t-tests comparing horses with wider inside and wider outside groups are shown.

Our results suggest that horses with wider inside forehooves exhibited movement patterns associated with increased loading of the inside limb, while those with wider outside fore-hooves showed movement patterns with increased loading with the outside limb. These findings suggest that the limb with the wider hoof experiences greater loading. Overall, this provides insights into the connection between training direction, hoof asymmetry, movement patterns, and limb loading, with implications for injury risk and training adaptations. Specifically, it appears that directional training and racing are consistent with a consistently increased force generation of the limbs on the inside of the circle. This is contrasting previous work with direct force measurements in trotting horses and suggests that different mechanisms are at work across gaits.

**Surface and Lungeing Direction and Movement Asymmetry (WP3):** WP3 focused on movement asymmetry in Thoroughbred racehorses during circular movement on different surfaces. Data from 30 horses (straight-line trot-ups, circular lungeing on turf and sand surfaces) were analysed. For the between-surface analysis, we compared movement asymmetry (UpDiff, MinDiff and MaxDiff) from horses trotting on turf and sand in a straight line using multiple paired t-tests. Significant differences were found in vertical movement asymmetry at the head (MaxDiff) and withers (UpDiff) (Figure 3), suggesting more pronounced head asymmetry on turf and more pronounced withers asymmetry on sand. No significant difference was observed for the pelvis ( $p > 0.05$ ).

This is interesting from a ‘compensatory’ aspect and for equine lameness workups, where withers movement differentiates between forelimb and hind limb lameness, suggesting that lameness workups should be conducted on different surfaces (even during straight line trot).

Comparisons between the three directions (straight, clockwise, and anticlockwise) on turf revealed that movement asymmetry varied across directions (repeated measures ANOVA, post-hoc pairwise comparisons). Significant differences were found for head and withers (Figure 4).

Specifically, during anticlockwise lungeing, we found a smaller difference in the range of head vertical displacement between the two halves of the stride (UpDiff) but larger limb compression (MinDiff) of the right (outside) limb compared to the straight. Larger limb compression of the right was also observed based on withers movement, together with a stronger left (inside) push-off. Observations at the withers were opposite during clockwise lungeing, with a larger limb compression of the left and a stronger right push-off. Pelvic movement differences were restricted to differences between clockwise and anticlockwise lungeing (Figure 5).

Given the complex interaction of circle and ‘lameness’ asymmetries reported from clinically lame horses, the movement patterns reported here specifically for Thoroughbred racehorses on turf, provide fundamental insights into primary and compensatory withers movement patterns in relation to predominant racing direction. The subtle differences between head and withers movement changes are important for creating Thoroughbred-specific guidelines.

In summary, this project has improved understanding about directional training, surface type, and hoof shape asymmetries and their effects in Thoroughbred racehorses. By pinpointing how these asymmetries appear under different exercise conditions, our research offers novel insights for clinical assessments in the form of Thoroughbred-specific patterns during lungeing on different surfaces.

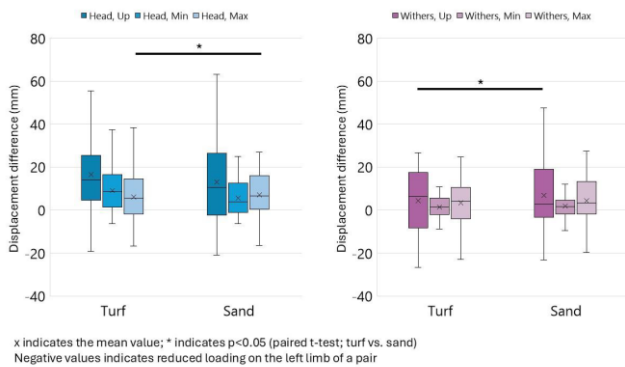


Figure 3. Boxplots of within-stride vertical displacement difference (mm) for head (left) and withers (right). Results of paired t-tests comparing between the turf and sand surface are shown.

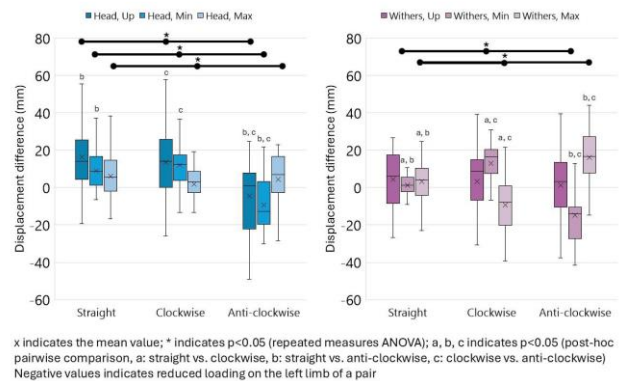


Figure 4. Boxplots of within-stride vertical displacement difference (mm) for head (left) and withers (right). Results of ANOVA and post-hoc pairwise comparison comparing between 3 directions (straight, clockwise and anticlockwise) are shown.

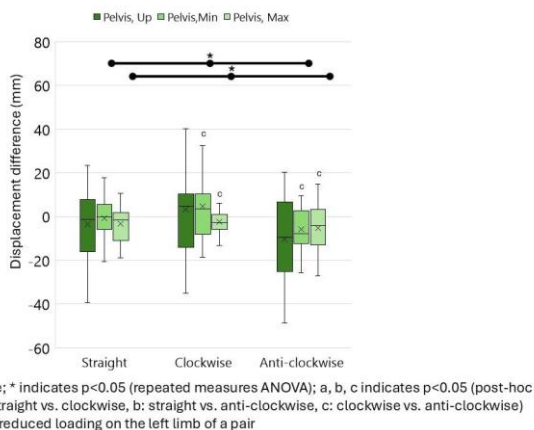


Figure 5. Boxplots of within-stride vertical displacement difference (mm) for the pelvis. Results of ANOVA and post-hoc pairwise comparison comparing between 3 directions (straight, clockwise and anticlockwise) are shown.

## Immunomodulatory effects of equine chondroprogenitor cells in an animal model of osteoarthritis

Principal Investigator:	Laurie Goodrich
Administering Institution:	Colorado State University, US
Amount Awarded:	USD 37,935 - Pump-prime Funding 2021
Project Period:	April 2022 - September 2023
Grant Reference No.:	PPF-2021-101639
Status:	Completed
Field of Research:	Musculoskeletal injury and disease / Immunopathology / Inflammation / orthopaedic disease / Orthopaedic regenerative medicine / Immunology

### Plain Language Summary

Osteoarthritis (OA) is a painful and debilitating joint disease with no permanent cure. Cartilage breakdown is a key feature of OA which results in pain, joint swelling and lameness. It is estimated that 60% of lameness in horses is OA-related, which translates to millions of horses being affected by this disease. Cartilage is a complex tissue to repair and, even after decades of research, the efforts to completely and permanently repair cartilage have been unsuccessful. “Stem cells” have recently become popular as a strategy to repair cartilage in patients with OA, most commonly using cells collected from bone marrow or fat (Mesenchymal Stromal Cells; MSCs). Although they have some advantages in healing injured cartilage, the effects are short-lived, and the repair tissue deteriorates over time. Recent research also shows that MSCs primarily act as a ‘signaling cell’, producing anti-inflammatory factors to help in healing and lessen pain rather than directly causing repair of the damaged cartilage. We have found a particular stem cell population (Articular ChondroProgenitors; ACPs) in normal joint cartilage that has several advantages, making it a superior option to MSCs as a stem cell for cartilage repair. Unlike bone marrow MSCs, they have the ability to divide and expand in culture long-term without loss of their cartilage-forming ability. In published studies and our work using horse chondroprogenitor cells, ACPs show the ability to suppress inflammation under cell culture conditions at similar levels as MSCs. Indeed, our present *in vitro* work confirmed that pro-inflammatory molecules were decreased by ACPs to the same extent as MSCs; excitingly, ACPs were also able to drive an increase in expression of anti-inflammatory mediators to a greater extent than MSCs. We also explored whether ACPs have anti-inflammatory and immunosuppressive properties in a whole living organism. To accomplish this, we injected horse ACPs into the joints of mice with OA and evaluated clinical and histopathologic outcomes. Of note, voluntary mobility parameters confirmed an improvement in disease symptoms. Further, histopathology confirmed an increased healing by ACPs in comparison with MSCs. With the significant advantages that ACPs offer, an understanding of their immunosuppressive properties that will increase their utility as a promising cell source for the treatment of OA. Our findings increase the support for use of ACPs as a cell-based product or their secreted molecules as a cell-free product for cartilage therapy.

### Key Findings and Outcomes of the Project

Osteoarthritis (OA) and the associated cartilage changes can be debilitating, resulting in lameness and functional disability in horses. Articular cartilage has a limited ability for intrinsic repair after injury and despite continuously evolving surgical and therapeutic strategies, success in regenerating the native articular cartilage remains elusive. A popular yet sub-optimal cell-based therapy involves the implantation of autologous chondrocytes (autologous chondrocyte implantation; ACI) or stem-like cells (e.g., mesenchymal stromal cells; MSCs). However, these approaches fail to recreate the native stable articular cartilage phenotype and therefore, have little effect in slowing disease progression and achieving consistent long-term cartilage regeneration. In contrast to the stem cell types used to date, articular cartilage contains a population of progenitor cells with the ability to generate stable articular-like cartilage. These articular chondroprogenitors (ACPs) are highly clonable and expandable while retaining their chondrogenic potential, features that are not true of other stem cells such as MSCs. Therefore, ACPs represent a superior cell population for cell-based therapies for the treatment of cartilage defects.

Therapeutic effects of MSCs have been increasingly attributed to their immunomodulatory properties, which are mediated by paracrine secretion of cytokines and growth factors that induce regenerative effects in the damaged tissues. Human ACPs have been shown to exhibit a strong immunomodulatory behaviour comparable to MSCs in an *in vitro* model of inflammation. Our central hypothesis was that articular

chondroprogenitor cells possess immunomodulatory properties *in vivo* that are advantageous for the treatment of osteoarthritis. We addressed this hypothesis by testing the immunomodulatory effects of ACPs after intra-articular injection in the DMM (destabilization of the medial meniscus) mouse model, a well-established and highly clinically relevant small animal model of post traumatic osteoarthritis (PTOA). Furthermore, we examined the immunomodulatory properties of the ACP-secretome, in comparison with the MSC-secretome in *in vitro* studies. The unique features of clonability and a stable cartilage phenotype over long-term culture make ACPs an ideal progenitor cell source for cell-based therapies for treatment of cartilage defects. This investigation of the immunosuppressive properties and trophic functions of ACPs provides critical knowledge to enhance their utility as a therapeutic target for healing damaged cartilage, particularly in inflammatory or immune mediated conditions. Importantly, the information obtained from this study offers preliminary data for detailed *in vivo* investigations in large animal models and will facilitate future therapies using ACPs and/or their secreted factors.

Specific aim 1 – To delineate the *in vivo* immunomodulatory properties of equine ACPs in a murine model of OA: ACPs and bone marrow-derived MSCs were isolated from horses and expanded in culture. Cells or vehicle control were intra-articularly administered into one hind limb knee of mice in each group, with the contralateral limb serving as a control. At 8-weeks following surgery, the three treatment groups were compared by clinical and histological evaluation of joints. Outcome measures included joint pathology and mobility/pain behaviors. Briefly, treatment with either MSCs or ACPs were superior to the vehicle control group at 2 weeks post-injury for total distance traveled and mean speed. Further, both treatment groups demonstrated significant improvements by 8 weeks post-injury in regards to average speed while mobile, time spent outside of their security hut, and freezing episodes. Notably, histopathology completed to date revealed a significant improvement in OA (as determined by a lower score) by APCs relative to MSCs.

Specific aim 2 – To characterize the immunomodulatory secretome of equine ACPs: The immunomodulatory properties of the cell secreted factors were compared by cytokine analysis of cell-conditioned media. Both MSCs and APCs decreased key inflammatory mediators tumor necrosis factor (TNF) and interleukin-1beta (IL-1beta); however, only APCs significantly increased the anti-inflammatory cytokine IL-10.

### **Investigation of equine fetlock joint immunopathology and the immunomodulatory effects of intra-articular therapeutics**

Principal Investigator: Heidi Reesink  
Administering Institution: Cornell University, US  
Amount Awarded: USD 186,055 - Research Training Scholarship 2021  
Project Period: January 2022 - December 2025  
Grant Reference No.: RTS-2021-101496  
Status: [Active](#)  
Field of Research: Musculoskeletal injury and disease / Immunopathology / Inflammation / orthopaedic disease / Orthopaedics / Immunology

The normal joint is composed of cartilage, an inner synovial membrane, and bathed in synovial fluid, along with additional supporting structures that surround the joint. Repetitive stress injury is a common cause for joint disease leading to osteoarthritis, which is commonly seen in racehorses. Osteoarthritis ultimately leads to joint inflammation, degeneration of the cartilage layer, bone proliferation along the joint edges, and alterations in the synovial fluid and synovial membrane layers. This inflammation and degeneration can lead to pain and reduced mobility, and is one of the most common causes for lameness in the horse. There is much unknown still about the immune cells participating in and propagating osteoarthritis, particularly in the horse. Our current therapeutic options can help mitigate the clinical signs of osteoarthritis for short periods of time but are unable to stop the development or progression of osteoarthritis.

Synovial fluid is composed of many proteins, all with a role in maintaining normal joint health. Lubricin is a protein present in synovial fluid with a primary role in boundary lubrication, or maintaining a friction-free surface within the joint at the cartilage surface; however, lubricin appears to have additional roles in the joint beyond lubrication. Lubricin acts as an anti-inflammatory on several types of immune cells that contribute to the development and progression of osteoarthritis. Numerous studies in rodents showed treatment with lubricin

prevented cartilage degeneration in a model meant to induce osteoarthritis. These anti-inflammatory and chondroprotective effects make lubricin a promising therapeutic target in managing osteoarthritis. The function of lubricin depends on the sugars attached to it (glycosylation), and this sugar profile changes in osteoarthritis in humans and horses.

In aim 1 we will evaluate immune cell populations in cartilage, synovial fluid, and synovial membrane in healthy and osteoarthritic equine joints. Flow cytometry will be used to identify various types of immune cells, including M1 and M2 polarized macrophages, T cells, B cells, NK cells, neutrophils, and dendritic cells. The cell populations will be compared between healthy and osteoarthritic joints, as well as with disease severity. In aim 2 the glycosylation of synovial fluid lubricin will be evaluated and compared to the immune cell populations gathered in aim 1. For aim 3 cartilage and synovial membrane will be treated with lubricin, triamcinolone, or platelet rich plasma; the last two being common articular treatments used to manage osteoarthritis inflammation and pain. Cultures will be activated to induce inflammation, and the effects of treatment on inflammation, macrophage polarization, and cartilage degeneration evaluated.

The long-term objectives are to identify immune cell populations present in equine joints with osteoarthritis to improve the treatment of joint disease. Lubricin shows potential in early studies as a disease-modifying therapeutic, and evaluation of the anti-inflammatory and chondroprotective effects will identify the suitability of lubricin as a treatment option. The glycosylation profile of lubricin in joint disease may provide a simple marker that can identify horses with early osteoarthritis to allow for prompt treatment prior to the development of more severe disease.

### **Mathematical modelling of metacarpal subchondral bone adaptation, microdamage, and repair in racehorses**

Principal Investigator:	Peta Hitchens
Administering Institution:	The University of Melbourne, Australia
Amount Awarded:	AUD 369,702 - Major Research Grant 2023
Project Period:	March 2024 - March 2026
Grant Reference No.:	MRG-2023-231012
Status:	<b>Active</b>
Field of Research:	Musculoskeletal injury and disease / Basic science of musculoskeletal system / Associations between training methods and risk of disease and injury / Orthopaedic disease

*Brief Summary: Injuries in racehorses occur when microdamage accumulates faster than the repair mechanism removes damage. By generating mathematical models representing subchondral bone adaptation, microdamage and repair, we can identify and develop training and management practices that reduce the incidence of racehorse injury, and improve outcomes in horses that have sustained injuries.*

Bone injuries in racehorses occur when microdamage, generated by repeated loading of the skeleton during galloping exercise, accumulates faster than the inbuilt repair mechanism of bone can remove the damage. The resultant injuries include fractures, both catastrophic and non-catastrophic and subchondral bone injuries which are common and cause poor performance, pain and lameness.

Although various risk factors for musculoskeletal injury in racehorses have been studied, they have not proven useful for early detection of injury or impending onset of conditions resulting in poor performance or catastrophic injury. And advances in diagnostic imaging have meant that pre-fracture pathology can be detected, but the widespread use of advanced imaging for the whole skeleton on a regular basis is not practical. Prevention of musculoskeletal injury through understanding how to manage a horse's workload appropriately is likely to have more universal application and therefore success. With the advent of technology that enables the accurate monitoring of galloping speed and distance, real time assessment of individual horse workloads is now achievable.

However, the relationship between workload and injury is complex with studies showing both too much and too little training and racing can lead to fracture. This is because of the complex relationships between bone

adaptation, damage accumulation and damage repair. Cross-sectional studies provide excellent baseline data for understanding such complex processes but only by modelling these processes can we hope to understand and predict them accurately.

We have previously developed a mathematical model of bone adaptation in Thoroughbred racehorses that approximates existing Thoroughbred racehorse training and rest regimens where bone adapts to training after about 14 weeks, but de-adapts in response to rest much faster, in 10 weeks or less. To further develop this model, we will account for bone microdamage accumulation and its repair.

This improved model will help us to better understand the process of bone adaptation, microdamage, and repair in racehorses so that we can assess and develop training strategies that reduce the risk of racehorse injury. This method is an ethical means of assessing the biological effect of changes prior to recommending and implementing lower risk training programs to the wider racehorse trainer community.

### **Mesenchymal stem cell licensing for improved tendon healing**

Principal Investigator:	Lauren Schnabel
Administering Institution:	North Carolina State University, US
Amount Awarded:	USD 164,640 - Major Research Grant 2022
Project Period:	June 2023 - May 2025
Grant Reference No.:	MRG-2022-100050
Status:	<a href="#">Active</a>
Field of Research:	Clinical Science and orthopaedics / Musculoskeletal biology, physiology and pathophysiology / Diagnosis and management of musculoskeletal disease and injury / Immunology / Inflammation / Immunopathology

Superficial digital flexor tendon (SDFT) injuries occur frequently in athletic horses and are particularly debilitating and costly due to both their extensive healing time and their high re-injury rate (50% to 70%). Rehabilitation from a typical tendon injury takes at least 6 months and often up to 1 year for full return to work. A re-injury during or after this rehabilitation period is devastating to any horse's career, but is especially devastating to that of a racehorse with a limited age and time frame for peak performance. SDFT injuries in racehorses can also negatively affect their ability to be adopted out after their racing career due to concerns about their performance ability in a second career. As such, SDFT injuries have a major impact on equine welfare.

SDFT injuries are characterized by tearing of fibers within the center of the tendon commonly referred to as core lesions and most often affect the forelimb SDFT at the level of the mid-third metacarpus. Current treatment strategies include rest, non-steroidal anti-inflammatory drugs (NSAIDs), controlled rehabilitation, and administration of local regenerative therapies, all in an effort to improve healing and return the horse to its previous level of competition. Mesenchymal stem cell (MSC) therapy has been shown to be effective at improving tendon architecture and also reducing reinjury rate in horses. Historically, MSCs have been administered following resolution of inflammation due to the extended time to diagnosis of tendon injury combined with the time required to isolate and expand patient-specific, or autologous, MSCs for therapy. However, recent work has revealed that the secretion of growth factors and immunomodulatory cytokines by MSCs needed for optimal healing can actually be enhanced through exposure to inflammation, termed MSC licensing. For these reasons, our group has been working diligently to characterize the tendon inflammatory environment post-injury and to assess how this tendon inflammatory environment affects MSC gene and protein expression. To date, we have characterized the cytokine environment in acute tendon injury with novel ultrafiltrate probes implanted in surgically induced core lesions of the SDFT. More recently, we demonstrated that licensing of MSCs with the inflammatory cytokine IL-1B and the immunomodulatory cytokine TGF-B2 markedly increases the expression of key genes related to tendon healing and are now investigating protein expression levels.

The purpose of this current proposal is to evaluate the ability of IL-1B and TGF-B2 licensed MSCs to improve tendon healing with the hypothesis that licensed MSCs will significantly enhance tendon healing compared to naïve MSCs in terms of improved lameness and ultrasonographic assessments in addition to biomechanics

and histologic evaluation. If our hypothesis is correct, the findings of this proposal would substantially alter clinical application of MSC therapy with the potential to markedly improve treatment outcomes and improve racehorse welfare by preventing early retirement or euthanasia and allowing them to more easily find homes after racing as sound horses with the ability to have a second career.

### **Multiscale modeling of subchondral bone fatigue injury at the equine fetlock joint**

Principal Investigator: W. Brent Edwards  
Administering Institution: University of Calgary, Canada  
Amount Awarded: CAD 136,042 - Research Training Scholarship 2023  
Project Period: May 2024 - April 2028  
Grant Reference No.: RTS-2023-233010  
Status: **Active**  
Field of Research: Musculoskeletal injury and disease / Basic science of musculoskeletal system / Strategies to prevent musculoskeletal disease and injury / Imaging / Orthopaedic disease

#### Publication:

Andrew Koshyk, Andrew J. Pohl, Colin R. Firminger, W. Brent Edwards, Probability of fatigue failure and minimum sample size requirements for cyclically loaded bone, *Journal of the Mechanical Behavior of Biomedical Materials*, Volume 169, 2025, 107061, ISSN 1751-6161, <https://doi.org/10.1016/j.jmbbm.2025.107061>.

*Brief Summary: This research is focused on the underlying mechanisms of bone fracture in Thoroughbred racehorses and directly aligns with the primary objectives of the Foundation including, but not limited to, efforts to reduce the incidence of disease and injury in racehorses while training, racing, and in retirement.*

The equine fetlock joint is one of the most commonly injured sites among racehorses, with racing relating injuries at this location being a significant source of lost training days and morbidity. Throughout the course of a season, young racehorses subject their limbs to repetitive, high intensity loads during high-speed racing and training sessions. Over time, this can result in damage to the bones at the fetlock joint and if this damage continues to accumulate in response to cumulative bouts of activity, it can eventually progress to a fracture.

The objective of this research is to model the equine fetlock joint and investigate how a variety of factors at multiple length scales contribute to the development of these injuries. This will be accomplished through a combination of advanced medical imaging, biomechanical testing, and computational modelling.

Understanding the underlying mechanisms of how these fractures occur will aid in the development of more effective training strategies to reduce the incidence of these injuries. The results of this work will provide a fundamental understanding of the development of these injuries and ultimately contribute to the prevention fractures in racehorses.

## Prediction of limb injury using inertial sensor data from Thoroughbred racehorses training and racing

Principal Investigator: Chris Whitton  
Administering Institution: The University of Melbourne, Australia  
Amount Awarded: AUD 570,226 - Major Research Grant 2024  
Project Period: February 2025 - February 2028  
Grant Reference No.: MRG-241012  
Status: [Active](#)  
Field of Research: Musculoskeletal injury and disease / Strategies to prevent musculoskeletal disease and injury / Early diagnosis of musculoskeletal disease and injury / Wearable Device

*Brief Summary: This project addresses two key Foundation's objectives: Reducing the incidence of disease and injury in racehorse while training and racing. And Developing management practices that improve the health and welfare of racehorses while training and racing.*

Deaths of racehorses are a major threat to the racing industry. Limb injuries are the most common reason for deaths of racehorses at the track. Less severe forms of limb injury are extremely common, cause substantial suffering and are therefore an important welfare issue. Prevention of limb injuries is challenging because pre-race veterinary examinations rarely show abnormalities. New preventative methods need to be practical and scalable so that all horses can benefit. The application of inertial sensor technology has already been shown to fulfil this with all horses in Tasmanian racing wearing sensors for the past 11 years and the Equimetre (Arioneo) has proven very promising to generate relevant data from training gallops. Therefore, such technology shows great potential to solve one of the most pressing and challenging problems in the racing industry.

This project aims to develop a combination of appropriate predictive statistical models from inertial sensor data collected during training and racing to allow prediction of the likelihood that a horse will develop a limb injury prior to its occurrence so that preventative strategies can be implemented. The project will further develop two approaches to speed and distance data that have to date shown promise, despite utilising incomplete data sets; 1) racing sensor data and 2) training speed and distance data without sensors. We now have access to wearable sensor data for all horses in training with Victoria's largest racehorse training business as well as sensor data from racing on all Victorian metropolitan racetracks. This more complete data will allow refinement of the models followed by implementation and testing to ensure they work. Measuring speed and stride data in the final stages of races has allowed detection of horses developing limb injuries for on average 5 races prior, while calculating the rate of accumulation of high-speed load on the skeleton based on our knowledge of bone fatigue damage is showing promise to detect horses at risk even earlier when analysing racing data or training data separately.

The analysis will occur over three stages: (1) utilisation of a joint modelling method (combination of two different types of model) to monitor how changes in speed and stride data over time are associated with injury; (2) modelling of the association between the accumulation of bone fatigue and injury; and (3) application of the models developed in stages 1 and 2 to predict injury prospectively.

The results of this research have the potential to be adopted widely in the racing industry by both trainers and regulators. The ability to identify horses at risk of limb injuries early will enable training modifications to be implemented to avoid future injury thus improving horse welfare and jockey safety.

## Real-time risk prediction for thoroughbred racing at The Hong Kong Jockey Club

Principal Investigator: Tim Parkin  
Administering Institution: University of Bristol, UK  
Amount Awarded: GBP 30,353 - Major Research Grant 2021  
Project Period: January 2022 - July 2023  
Grant Reference No.: MRG-2021-101678  
Status: [Completed](#)

Field of Research: Epidemiology / Predictive modelling / Welfare, studies into management practices that impact the welfare of racehorses

### Plain Language Summary

These analyses show the benefit of being able to access and utilise historical veterinary data for the identification of risk factors associated with deleterious outcomes during racing. For both models the estimated degree of accuracy of prediction (which therefore opens up the potential for prevention) is much greater than seen before in any models developed in this particular area of research, in this or other racing jurisdictions around the world.

The range of risk factors identified confirm the complex nature of these outcomes and indicate, as in most other equivalent models, that there are multiple factors that all contribute relatively small amounts to the overall risk at each start. However, when used together to develop a complete risk profile they result in a significant difference in risk between those making starts with the highest and lowest risk. Our aim is to be able to provide these risk profiles prior to pre-race checks so that veterinary surgeons conducting those checks are able to use this information when making decisions about whether to permit horses to race or not.

### Key Findings and Outcomes of the Project

Multivariable Bayesian generalized additive multilevel models have been developed for two outcomes: post-race lameness and unacceptable performance in races held at the HKJC.

#### Post-race lameness (LA)

The final model identified 14 risk factors statistically significantly associated with LA and indicated possible association for seven additional factors. The factors can be grouped into three broad categories: race- and racing history-related, veterinary history-related, and training history-related risk factors.

Within the first category, LA was associated with the month of race during a racing season. The likelihood of LA changed little between September and January, after which it began to increase steeply until the end of racing season in July. The predicted probability of LA in July was approximately two times greater than in September to January. LA was associated with racecourse and track; compared to starts made at Sha Tin on turf (ST-T), starts made at Happy Valley on turf (HV-T) or Sha Tin on all-weather track (ST-AW) were at increased odds of LA (OR 1.17, 95% CI 1.01 – 1.36 and 1.65, 1.39 – 1.96, respectively). LA was also associated with race distance – in two ways. First, longer distance of the current race was associated with increased odds of LA, with an OR of 1.08 (1.05 – 1.12) per each additional 100 metres. Second, starts made by horses with longer average distance in their previous races (i.e., usually racing over longer distances) were at reduced odds of LA. The predicted probability of LA decreased steeply between 1,000 and approximately 1,500 metres of average distance in previous races, after which the decrease continued at a reduced rate, and at 1,800 metres was approximately half of that at 1,000 metres. Starts made by horses ridden by apprentice jockeys were less likely to result in LA (0.85, 0.72 – 1.00). LA was also associated with the final position in the previous race. Horses that placed lower in the previous race had reduced odds of LA following the current race, with an OR of 0.95 (0.93 – 0.97) per each additional place away from the first place.

Concerning veterinary history-related risk factors, LA was associated with receiving phenylbutazone treatment; starts made by horses that received phenylbutazone since the day following the day of the previous race were at increased odds of LA (1.17, 1.00 – 1.36). LA was also associated with having an X-ray and receiving intra-articular corticosteroids (IACS) or receiving IACS alone since the previous race. Starts made by horses that had an X-ray and IACS or IACS alone since the previous race were at increased odds of LA (2.21, 1.85 – 2.64 and 1.67, 1.27 – 2.14, respectively). Importantly, LA was also associated with the frequency of having an X-ray and receiving IACS and the frequency of receiving IACS alone. Starts made by horses that had an X-ray and IACS or received IACS alone more frequently between their past races were associated with a substantially increased likelihood of LA. The model also indicated potential associations between LA and receiving meloxicam treatment since the previous race (1.26, 0.99 – 1.66) and receiving meloxicam repeatedly (1.10, 0.99 – 1.28), but these were not statistically significant at a 95% CI level.

Concerning training history-related risk factors, LA was associated with the number of trot workouts a horse had in the last 14 days before the race and gallop workouts in the last 15 – 28 days before the race. The predicted probability of LA decreased with an increasing number of trot workouts, reached the minimum at around nine workouts, and then began to rise until 14 workouts per the 14-day pre-race period. Starts made by horses with more gallop workouts 15 – 28 days before the race were at reduced odds of LA, with an OR of 0.94 per each additional gallop workout (0.88 – 1.00).

With training data, the final model's area under the receiver operating characteristic curve (AUC-ROC) was 0.85, which is approximately 15% better than most previous models from other jurisdictions (lacking complete veterinary and training history data). The area under the precision-recall curve (AUC-PR) was 0.11 – approximately 7.3 times greater than that of a baseline intercept-only model.

### Unacceptable performance (UP)

The final model identified 13 risk factors statistically significantly associated with UP and indicated possible association for three additional factors.

UP was associated with the month of race during a racing season; the predicted probability of UP in July was approximately 1.6 times greater than in December to March. UP was also associated with racecourse and track; compared to starts made at ST-T, starts made at HV-T and ST-AW were at decreased (0.83, 0.71 – 0.99) and increased (2.23, 1.92 – 2.61) odds of UP, respectively. UP was also associated with race distance; longer distance of the current race was associated with increased odds of UP, with an OR of 1.31 (1.27 – 1.34) per each additional 100 metres, while starts made by horses with longer average distance in their previous races were at reduced odds of UP. The predicted probability of UP decreased steeply between 1,000 and approximately 1,300 metres of average distance in previous races, after which the decrease continued at a reduced rate, and at 1,800 metres was approximately one-fourth of that at 1,000 metres. Each additional pound carried by a horse (1.02, 1.01 – 1.03) and each additional year of age at the time of the first start in a career (1.17, 1.05 – 1.28) were associated with increased odds of UP. Notably, horses that placed at the top or bottom of the field in the previous race and/or on average in their previous races (i.e., usually) were at substantially increased odds of UP compared to those positioned in the middle. Starts made by horses that had bronchoscopy before their first race start were at increased odds of UP (1.19, 1.00 – 1.42).

With training data, the final model's AUC-ROC was 0.86. AUC-PR was 0.16 – approximately 9.2 times greater than that of a baseline intercept-only model.

For both models, prediction with new data shows promising results supporting their usefulness for real-life pre-race risk profiling as an additional decision-making tool.

## Relationship between Thoroughbred racing and training workloads and the fatigue life of equine subchondral bone

Principal Investigator:	Chris Whitton
Administering Institution:	The University of Melbourne, Australia
Amount Awarded:	AUD 243,404 - Major Research Grant 2021
Project Period:	April 2022 - September 2024
Grant Reference No.:	MRG-2021-101717
Status:	Completed
Field of Research:	Epidemiology / Anatomy and physiology / Musculoskeletal injury and disease / Strategies to prevent musculoskeletal disease and injury / Association between training methods and risk of disease injury

### Plain Language Summary

Previous predictive models of musculoskeletal injuries in racehorses have treated injury as a yes or no outcome – that is, the horse is either injured or not injured. However, damage accumulates over time even though an injury is only recognised when a threshold of damage is reached. For bone this is the process of bone material fatigue, the damage that accumulates through repeated loading of the skeleton when galloping. This is

evidenced by the numerous cases of catastrophic injury in horses where pre-existing pathology is found at postmortem.

We built on our previous research, which used only racing data, to estimate bone damage accumulation. In this study, we combined records of both racing and training workloads to estimate bone fatigue accumulation. To achieve this, we used ten years of training and racing data from Thoroughbred racehorses actively training and racing in Hong Kong. From this data we implemented published equations to estimate the proportion of an individual horse's bone fatigue life that has been used up, an indication of the relative impact of each horse's training history. Following this we investigated how well these workload estimates predicted musculoskeletal injury.

We were able to determine and assess differences in the relative impact of workloads for different types of training events (slow work, fast work, barrier trials) as well as racing events on the fatigue life of bone based on speed, distance, and other training data. We found that when observing a single event, accumulated bone fatigue damage was greatest for races, followed by barrier trials, fast work, then slow work. This is in line with what we know about the relationship between speed and load on the limb, whereby faster speeds result in greater loads thereby resulting in fewer stride cycles being tolerated before the potential occurrence of a bone fatigue injury.

However, over the career of the horse, as well as per race preparation, the total cumulative percentage fatigue life was higher for all slow and fast workouts as well as all training events collectively (including barrier trials) compared to races. Fast work was the greatest contributor to bone fatigue accumulation. Also, horses that had higher training volumes did not necessarily have higher corresponding racing volumes demonstrating that solely assessing racing workloads may not serve as a sufficient representation of total work volume in determining injury risk. Additionally, the contribution of bone fatigue accumulation changes as a horse's career progresses where, at older ages, the contribution of races towards the percentage fatigue life accrued overtakes that of slow work.

We demonstrate the importance of monitoring training workloads in determining injury risk. The strength of this approach is that rather than only predicting the endpoint to musculoskeletal injury per previous epidemiological studies, we can map the development of injuries over time, which will allow for much earlier intervention.

### **Key Findings and Outcomes of the Project**

Modelling the accumulation of bone fatigue using racing stride data has allowed for a deeper understanding of loading on bone in Thoroughbred racehorses. Quantifying additional loads the horse is subject to during training allows for a more complete understanding of bone fatigue accumulation and its effect on injury.

In this project we quantified additional loads racehorses are subject to during training and estimated the total accumulated bone fatigue incurred during both training and racing events.

This was achieved using a dataset containing training and racing histories of Thoroughbred horses (n=5,539) actively training and racing in Hong Kong from 5 September 2010 to 14 July 2021. We used published equations to calculate the percent fatigue life (%FL) accumulated per racing and training event, per preparation, and per career. Associations between event type (slow work, fast work, barrier trials, and races) and accumulated fatigue life were assessed at both the horse and trainer level, along with their relationship to injury.

Key findings:

- Accumulation of %FL per training event was lowest for slow work (0.4%), followed by fast work (3.2%), barrier trials (8.2%), then races (10.1%).
- %FL accumulation increased with longer race distance.
- The percentage contribution to the total fatigue life accumulated over horses' race preparations was highest for fast work (39.6%), followed by slow work (29.1%), races (17.8%), then barrier trials (5.6%).

- Training events contributed a lower proportion of fatigue accumulation per event compared to races, but over a preparation or career they accounted for a greater proportion of fatigue life due to the greater frequency or volume of work.
- Younger horses (<5 years of age) accumulation of fatigue was primarily attributed to fast work followed by slow work. However, as a horse ages, the contribution of races towards the percentage fatigue life accrued overtakes that of slow training. This is due to the increase in the number and volume of races undertaken by horses as they progress in their careers.
- Training and racing workloads varied significantly between trainers.
- Acute (per event), intermediate (per preparation) and chronic (per career) rates of fatigue accumulation are key contributors to risk of musculoskeletal injury.

#### Key outcomes:

- Developed a method to determine when racehorses are accumulating bone damage at high rates based on training and racing loads.
- Determined horse- and trainer-level factors influencing bone damage accumulation.
- Training in the application of advanced modelling methods for postdoctoral researchers Dr Adelene Wong and Dr Ashleigh Morrice-West.
- Collaborative relationship established with Dr Euan Bennet from the University of Glasgow.
- Epidemiological training and successful completion of the DVM student research project by Dr Will Height.
- Presentation at the IFHA Global Summit on Equine Safety & Technology by Prof. Whitton and A/Prof. Hitchens, of which this study contributed knowledge on accumulation of bone damage during racing and training.

### Risk assessment for condylar stress fracture in the racing thoroughbred

Principal Investigator:	Peter Muir
Administering Institution:	University of Wisconsin-Madison, US
Amount Awarded:	USD 338,278 - Major Research Grant 2022
Project Period:	April 2023 - July 2026
Grant Reference No.:	MRG-2022-100008
Status:	Active
Field of Research:	Musculoskeletal injury and disease / Improved methods of detection of structural fatigue-related injury in racehorses / Imaging / Orthopaedic disease

#### Publications:

- ▶ Muir, P. and Whitton, R.C. (2024), Injury prevention in Thoroughbred racehorses. *Equine Vet J*, 56: 386-388. <https://doi.org/10.1111/evj.14077>
- ▶ Irandoust Soroush, Whitton Chris, Henak Corinne and Muir Peter. 2025 Tuning and validation of a virtual mechanical testing pipeline for condylar stress fracture risk assessment in Thoroughbred racehorses. *R. Soc. Open Sci.* 12:241935. <https://doi.org/10.1098/rsos.241935>

Injuries to the Thoroughbred racehorse that lead to euthanasia are termed catastrophic; 80% of such injuries involve the locomotor system. Condylar stress fracture represents ~25% of catastrophic injury. Race performance after surgical treatment of Thoroughbreds with condylar fracture is often disappointing. There is a critical unmet need for development of clinically relevant methods differentiating dangerous from safe lesions given the high prevalence of stress injury to fetlock bone. With use of standing computed tomography (sCT), fetlock bone injury can be readily identified. Our long-range goal is to reduce the incidence of catastrophic injury in racehorses by sCT fetlock screening, which can be performed without disruption to training.

The objective of this application is to advance identification of racing Thoroughbreds with concerning injury to fetlock bone that have a high imminent risk of serious injury. We aim to develop a fast, accurate, clinically relevant analysis method. Our hypothesis is that a patient-specific computer model, known as a finite element model, for analysis of fetlock sCT features will enable identification of horses with imminent risk of serious injury from condylar stress fracture. Our hypothesis is based on strong preliminary data. In this project, we will compare two complimentary approaches for building patient-specific models and compare their predictive

accuracy. This approach will rapidly optimize our computational approach for clinical screening, thereby yielding substantial reductions in serious injury in racehorses across the world and a strong rationale.

To accomplish our objective, we will pursue the following specific aims: (1) Identify horses with a high fracture risk using a 3D model of the fetlock joint to assess bone injury using an analytical method that considers cumulative cyclic loading of the joint and (2) Identify horses with a high fracture risk using a simpler and faster thin slice model of an oblique frontal plane slice of the joint surface. Under Aim 1, we will extend our validated 3D patient-specific modeling capabilities to include consideration of cyclic loading in the stress fracture risk analysis and analyze longitudinal sCT scans from Thoroughbreds beginning race training. Under Aim 2, we will analyze a simplified patient-specific 2D FE models of an oblique frontal slide of the joint surface and compare predictive performance of thin slice and 3D FE models. As a prelude to this project, we have designed and built a state-of-the-art commercially available sCT scanner for the standing horse that has enabled routine fetlock CT scanning clinically. The approach is innovative because it exploits the capabilities of sCT imaging systems. Regarding outcomes, we expect to develop a solid basis from which routine fetlock screening of large populations of Thoroughbreds with minimal risk and no disruption to training, thereby providing a high translational impact. Horses with concerning lesions and a high risk of imminent injury can be identified for personalized care. Clinical implementation of this work will save the lives of many racehorses. Routine implementation of preemptive longitudinal monitoring of horses in training will mean improved care for racehorses and better prevention of condylar stress fracture.

### Unravelling the genetic mechanisms underlying fracture risk in horses

Principal Investigator:	Debbie Guest
Administering Institution:	The Royal Veterinary College, UK
Amount Awarded:	GBP 164,850 - Major Research Grant 2021
Project Period:	November 2022 - October 2024
Grant Reference No.:	MRG-2021-101067
Status:	Completed
Field of Research:	Bone fracture / Genetics / Musculoskeletal injury and disease / Early diagnosis of musculoskeletal disease and injury / Genetics factors that influence risk of musculoskeletal disease and injury / Molecular biology / Orthopaedic disease

### Plain Language Summary

Bone fractures often occur in Thoroughbred racehorses and are the main reason for euthanasia on the racecourse. Fracture is a complex condition with both environmental (e.g. race conditions) and genetic (i.e. inherited) risk factors. The aim of this project was to identify changes in the DNA that are associated with fracture.

We used a technique called whole genome sequencing to read every single base in the DNA sequence of seven horses who had a fatal fracture and seven horses that never had a fracture. This technique identified over 12 million changes in the DNA sequences (variants) between the individual horses. By looking at where these DNA changes were located, how common they were in different breeds, what they were predicted to do and how many of the fracture cases and controls they were found in, we were able to narrow this down to 474 DNA variants of interest. These variants were then measured in 155 horses with fatal fractures and 206 control horses without fractures. We found that 25 of the variants were significantly associated with fracture.

A large proportion of these variants were located on chromosome 18 which has previously been associated with fracture risk. 14 of the variants were in coding regions of DNA and are predicted to affect how a protein works. 11 of the variants were in non-coding regions of DNA and are predicted to affect how much of a gene and subsequently, a protein, is produced. The majority of the genes and proteins that are affected by the variants do not currently have published links to bone and further work should identify if they have novel roles.

The identification of new DNA variants and genes that are associated with fracture will allow us to improve our genetic test to identify horses at high risk of fracture and lead to future research to understand why these horses are at increased risk so that we can develop new interventions to prevent fractures occurring. This will have a significant welfare benefit for Thoroughbred racehorses.

## Key Findings and Outcomes of the Project

In this project we identified new DNA variants that are associated with catastrophic fracture in Thoroughbred horses.

Catastrophic fractures due to bone overloading occur in Thoroughbred racehorses and are associated with both environmental and genetic risk factors. A previous genome wide association study (GWAS) identified single nucleotide polymorphisms (SNPs) across the genome that were associated with fracture risk <sup>1</sup>. A region of chromosome 18 that contains multiple genes with significant roles in bone, was the most strongly associated with fracture risk. In more recent work, we identified a functional SNP upstream of the *COL3A1* gene which was significantly associated with fracture<sup>2</sup>. However, no other causal DNA variants have been identified.

In this study, we performed whole genome sequencing (WGS) on seven fracture cases and seven fracture controls. This identified 12,224,941 variants across all genomes. The variants were filtered based on their effect, genomic location, minor allele frequency (MAF) and segregation between the cases and controls. A total of 474 variants were then used in MASSARRAY genotyping in a larger cohort of 155 fracture cases and 206 fracture controls. After quality control, 25 variants were found to be significantly associated with fracture. 11 of the variants were upstream of six different genes. 14 variants were mis-sense variants that were present within ten different genes (Table 1).

Variant	Chr	Effect	Gene
SNP_419 *	18	Upstream	COL5A2
SNP_423	18	Upstream	OSGEPL1
SNP_427	18	Upstream	PMS1
SNP_431	18	Upstream	C18H2orf88
SNP_462	18	Upstream	NEMP2
SNP_436	18	Upstream	INPP1
SNP_439	18	Upstream	INPP1
SNP_448	18	Upstream	INPP1
SNP_452	18	Upstream	INPP1
SNP_453	18	Upstream	INPP1
SNP_460	18	Upstream	INPP1
SNP_079	10	Missense	PPM1N
SNP_680	18	Missense	ICA1L
SNP_162	18	Missense	ICA1L
SNP_154	18	Missense	FSIP2
SNP_535	4	Missense	GTPBP10
SNP_035	4	Missense	STEAP4
SNP_398	16	Missense	SLC35G2
SNP_553	7	Missense	C3
SNP_323	1	Missense	FSCB
SNP_324	1	Missense	FSCB
SNP_037	4	Missense	MTERF1
SNP_578	10	Missense	ZNF180
SNP_164	18	Missense	ICA1L
SNP_166	18	Missense	ICA1L

Table 1. Variants significantly associated with fracture.

Of the 474 variants used in the genotyping, 75 were on chromosome 18 (15.8%). However, of the 25 variants that were significantly associated with fracture, 16 were on chromosome 18 (64%). This supports the results of the original GWAS study and implicates this chromosome in fracture risk.

Of the 25 fracture-associated variants, 19 variants conferred risk and four were protective (Figure 1).

Epistatic interactions were present between all of the upstream variants and the four mis-sense variants in *ICA1L* (Figure 2).

SNPs\_162, 164, 166 and 680 are all risk variants with very similar minor allele frequencies (MAFs) across different breeds. In Thoroughbreds, the MAFs are 0.11 or 0.12. The highest MAFs are observed in Morgan horses (0.27-0.36) and the lowest MAFs in Shetland ponies (0.01-0.04). The GERP conservation scores did not indicate high evolutionary constraint. SNP\_162 was not predicted to have a deleterious effect on protein function. However, SNP\_164, SNP\_166 and SNP\_680 were all predicted to have deleterious effects.

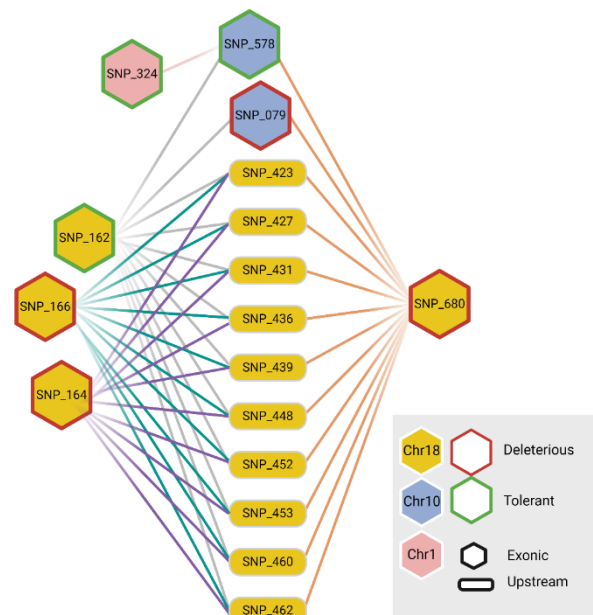
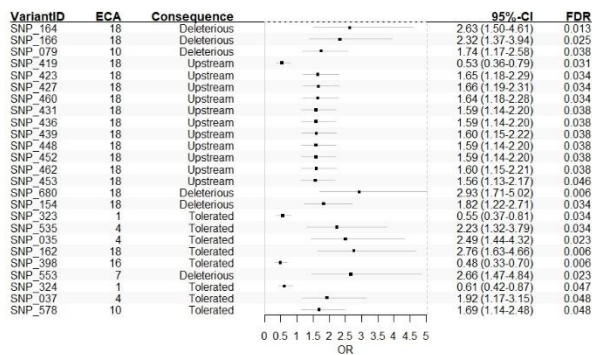


Figure 1. Forest plot showing the odds ratio (OR) and chromosome (ECA) for each variant. Figure 2. Epistatic interactions between the variants.

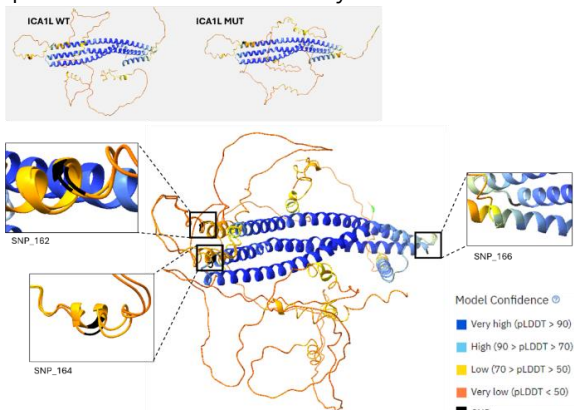
Using Alphafold we demonstrated that SNP\_162, SNP\_166 and SNP\_164 are predicted to affect alpha-helices at different points within the protein (Figure 3). SNP\_162 and SNP\_164 lie within the C-terminal domain, whereas SNP\_166 lies within the arfaptn homology domain. SNP\_680 would only be mis-sense in one transcript (ENSECAT00000120577) and the model for this transcript is very low confidence (data not shown). Alphafold models were created for all other proteins which contained putative mis-sense variants, but no major effects (e.g. truncated proteins) were found (data not shown).

Little is known about the function of *ICA1L*. It is expressed in equine bone tissue<sup>3,4</sup> and intronic variants are associated with appendicular lean mass and heel bone mineral density<sup>5</sup>. We have demonstrated that *ICA1L* is expressed during bone formation by human Saos2 cells and primary equine osteoblasts (Figure 4). We have recently started an MRes project to determine the role of *ICA1L* in bone cells *in vitro*.

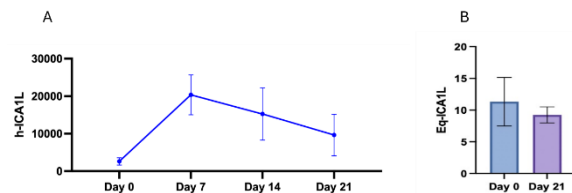
Of the other mis-sense variants, SNP\_535 is in the *GTPBP10* gene. Knock-out mice have significantly increased bone mineral content<sup>6</sup>, but the variant we have identified is not predicted to have deleterious effects on the protein. SNP\_553 is in the *C3* gene and is predicted to be deleterious to the protein. *C3* knock-out mice have delayed fracture healing<sup>7</sup>. None of the other mis-sense variants were within genes that had known associations with bone or fracture.

For the upstream variants, SNP\_419 was present upstream of *COL5A2*. *COL5A2* regulates the assembly of collagen fibrils<sup>8</sup> and is involved in the early stages of osteoblast differentiation<sup>9</sup>. SNPs\_436, 439, 448, 452, 453 and 460 are all upstream of *INPP1*. *INPP1* encodes the enzyme inositol phosphatase-1-phosphatase which is involved in phosphatidylinositol signaling. *INPP1* is expressed in equine bone tissue<sup>3,4</sup> and although its role has not been defined phosphatidylinositol signaling involved in many cellular processes in bone tissue. None of the other non-coding variants were upstream of genes that had known associations with bone or fracture.

For the upstream variants, our preliminary data demonstrates that some of the upstream SNPs lie in regions with promoter/enhancer activity.



**Figure 3.** Locations of the mis-sense SNPs on the ICA1L protein, as predicted using AlphaFold. The top image shows the overall wild-type (WT) structure compared to the structure of the mutated (MUT) protein with SNP\_162, SNP\_164 and SNP\_166. The bottom image shows the alignment of the WT and MUT protein structures with the SNPs enlarged. SNPs are shown in black, the other colours indicate the model confidence.



**Figure 4.** ICA1L is expressed during basal and osteogenic culture of A) Saos2 cells and B) equine osteoblasts. Expression of human (h) and equine (eq) ICA1L is shown normalized to either the ACTB housekeeping gene (for Saos2 cells) or the 18s rRNA gene (for equine osteoblasts). The relative levels of the expression can therefore not be compared between the cell types.

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## Use of mRNA expression and serum biomarkers as tools to monitor musculoskeletal adaptation in Thoroughbred horses returning to race training

Principal Investigator:	Allen Page
Administering Institution:	University of Kentucky, US
Amount Awarded:	USD 379,495 - Major Research Grant 2023
Project Period:	July 2024 - December 2026
Grant Reference No.:	MRG-2023-231025
Status:	<a href="#">Active</a>
Field of Research:	Musculoskeletal injury and disease / Strategies to prevent musculoskeletal disease and injury / Early diagnosis of musculoskeletal disease and injury / Molecular biology / Genetics

*Brief Summary: This project specifically addresses objectives: (i) of reducing the incidence of disease and injury in racehorses while training, racing and in retirement; and (ii) developing management practices that improve the health and welfare of racehorses while training, racing and in retirement; , in that the ability to predict and prevent lameness or poor performance will likely have consequential benefits in the reduction of risk for potentially fatal or career ending injuries, keeping more horses sound, improving horse welfare, and extending their racing careers.*

Our duty to care for and heightened public awareness of racehorses has led to increased pressure for additional research into racehorse well-being and injury prevention. An important area that requires dedicated research is in identifying and preventing non-fatal injuries given the direct link between previous lameness and/or being placed on a “vet list” and the risk of fatal injury. As such, a critical unmet need is the development of a sensitive and specific screening tool to pre-emptively identify horses at risk of injury.

Studies have demonstrated that racehorses returning following a layoff/spell are at an increased risk for injury as they return to training and racing. This is due to changes in bone that occur when the stresses of training and racing are removed. Racehorses being imported into Hong Kong provide an important opportunity to monitor these changes during reconditioning, where approximately 20% of these horses will experience an injury within the first 3-4 months following import. Based on our prior and ongoing work, we believe that messenger RNA (mRNA) expression analysis in combination with cortisol and bone biomarker measurement could provide a means for identifying racehorses whose skeletons are not coping with training and are at risk of injury such that intervention prior to injury may be possible.

As such, we hypothesize that messenger RNA expression analysis combined with cortisol and bone biomarker measurements can be utilized to monitor racehorses in training and identify those at risk for poor performance and/or injury. Weekly veterinary examinations and trainer feedback will identify horses with signs of injury/illness or performance concerns while additional records will be collected for analysis. This project will use regularly collected samples from 80 previously trained +/- raced Thoroughbred horses that undergo a substantial period of deconditioning during importation quarantine for Hong Kong, and monitor their weekly responses to retraining/reconditioning by measuring changes in mRNA expression, serum cortisol, and bone biomarkers.

Ultimately, this project will provide essential data to improve the welfare of horses not only entering Hong Kong, but across the globe. A critical step in this process is to better understand the responses of equine athletes to conditioning and reconditioning using existing tests that have proven useful for identifying fracture risk. Data collected during this project will serve as important building blocks for future projects as we strive for better methods to reliably detect and prevent injuries in Thoroughbred racehorses.

## Diseases associated with intensive training

### Applying novel multi-omic approaches to investigate the impact of training on airway immunity and molecular pathways underpinning MMEA and EIPH

Principal Investigator:	Scott Pirie
Administering Institution:	The University of Edinburgh, UK
Amount Awarded:	GBP 181,243 - Major Research Grant 2021
Project Period:	August 2022 - July 2025
Grant Reference No.:	MRG-2021-101925
Status:	Active
Field of Research:	Respiratory / Airway inflammation and haemorrhage / Respiratory health and disease / Identification of risk factors associated with respiratory disease / Genetics / Molecular biology / Immunology / Inflammation

This project will use state-of-the-art investigative approaches to improve understanding of the cause and mechanisms underpinning two highly prevalent, performance-limiting disorders of racehorses; namely, mild to moderate equine asthma (MMEA) and exercise induced pulmonary haemorrhage (EIPH). This is a necessary step in the development of more targeted preventative and treatment options for these disorders. Although a number of factors have been associated with MMEA, incomplete understanding of the precise cause and course of events underpinning this syndrome means that our current treatment options are limited. Similarly, while EIPH is associated with progressive narrowing of the veins draining the lungs leading to an increase in blood pressure within the microscopic blood vessels surrounding the air sacs, the additional and potentially pivotal contribution of inflammation at this site has received little attention. Further understanding of the mechanisms underpinning MMEA and EIPH offers potential to identify specific targets for treatment and preventative interventions.

We have demonstrated that training per se can alter the immune status of cells within the airway, with the potential to result in airway inflammation and/or susceptibility to infections. This was evidenced by changes in the genetic coding for (transcriptomics), or levels of a vast array of specific cell messenger proteins (proteomics) within samples derived from the airways of racehorses in training. As genetic coding for protein production does not always align with “end stage” protein production, this combined approach enables a more holistic assessment of the immune status of the lower airways and is more sensitive and specific than current methods used to “measure” inflammation (i.e. counting inflammatory cells). It also facilitates the “mapping” of specific immune and inflammatory pathways; effectively “joining the dots” between detected changes in groups of proteins and the processes which they control (e.g. inflammation, remodelling), potentially revealing appropriate interventional targets.

We hypothesise that:

- (1) race training results in an altered immune status of the lower airways, characterised by changes in genetic coding for, and/or production of, messenger proteins and that this combined approach will have superior sensitivity in detecting inflammation and greater specificity in identifying potential treatment targets than the currently adopted method of inflammatory cell counting;
- (2) this combined approach will reveal the key inflammatory pathways specific to different MMEA “types” (currently based on the predominant inflammatory cell), with the potential to inform more “MMEA type”-specific therapies; and
- (3) application of a state-of-the-art methodological approach (spatial transcriptomics) to lung tissue samples derived from EIPH cases (post mortem) will allow identification of the specific cells responsible for encoding the production of messenger proteins at the site of bleeding. This will help to elucidate the relative role of both specific cell types (e.g. blood vessels, lung cells) and specific pathways (e.g. remodelling, inflammation) in this complex disease and further scrutinise the link between airway inflammation and bleeding.

The methodologies applied to address the above hypotheses will provide a greater understanding of the mechanisms underpinning MMEA and EIPH and help to inform the development of more targeted treatment and prevention strategies.

### Defining a transcriptomic signature for equine recurrent laryngeal neuropathy

Principal Investigator: Richard Piercy  
Administering Institution: The Royal Veterinary College, UK  
Amount Awarded: GBP 189,144 - Research Training Scholarship 2021  
Project Period: April 2022 - October 2025  
Grant Reference No.: RTS-2021-101938  
Status: **Active**  
Field of Research: Upper airway disorders / Transcriptomics and molecular biology / Pathophysiology of Recurrent Laryngeal Neuropathy / neurological disease / Molecular biology / Genetics

Most, if not all, Thoroughbred horses are affected by a disease, known as 'roaring', that causes the death of nerve fibres that supply the horse's larynx ('voice box'), primarily on the left side of a horse's throat. Whilst most horses are not clinically affected, in the severest cases, horses cannot open their airway sufficiently when exercising due to the associated left sided larynx muscle weakness, meaning that their air intake is compromised and they perform poorly. These horses are either retired from racing, euthanased or they undergo surgical procedures artificially to tie open the airway, enabling sufficient air to enter the lungs during strenuous exercise. Unfortunately, the permanent alteration to the airway that results from this surgical procedure can result in food material entering the lungs during swallowing with associated lung infections and coughing. It is common also for the surgical procedure to fail. As such, roaring has substantial welfare and economic burden to horse racing worldwide.

Even though we have long recognised this disorder, there is remarkably poor understanding of the cause: genetic, environmental or acquired causes are all postulated. However defining the cause has proved exceptionally difficult due to anatomic and methodological constraints. We predict that improved understanding of the mechanisms that result in death of these nerve fibres will help in the search for the cause of the disease. Consequently, in this study we aim to shed light on the underlying mechanisms that result in cell stress within the very long nerves that supply the larynx by assessing the expression of genes within defined regions of the equine brain, where the nuclei of these nerve cells are located. We hypothesise that specific subsets of these nuclei, especially on the left side, express patterns of genes that will help define a disease mechanism for this enigmatic disorder. We will utilise a series of sophisticated molecular techniques examining tissues from horses with varying severities of the disorder with the aim, ultimately, of defining the reason why nerve cells die in affected horses; if successful, the results might then prompt investigation of alternative strategies for disease management, prophylaxis or treatment, with the ultimate goal of improving Thoroughbred welfare worldwide.

### Does Vitamin D deficiency have a role to play in Recurrent Exertional Rhabdomyolysis?

Principal Investigator: Charlotte Maile  
Administering Institution: University of Surrey, UK  
Amount Awarded: GBP 12,040 - Pump-prime Funding 2024  
Project Period: December 2024 - November 2025  
Grant Reference No.: PPF-242003  
Status: **Active**  
Field of Research: Musculoskeletal injury and disease / Strategies to prevent musculoskeletal disease and injury / Early diagnosis of musculoskeletal disease and injury / Metabolism

*Brief Summary: This project aims to reduce the incidence of disease and injury and improve veterinary care to achieve better outcomes in racehorses. This project aligns with the priority research topic on husbandry and management as vitamin D deficiency may be rectified through husbandry changes (access to pasture or dietary supplementation).*

Racing Thoroughbreds are hugely athletic animals with up to 55% of their body mass comprised of skeletal muscle. Vitamin D is essential for normal muscle function and in humans, a deficiency in vitamin D has been associated with various forms of muscle disease including those related to exercise. A predominant form of exercise-associated muscle disease in Thoroughbred horses is recurrent exertional rhabdomyolysis and this disease affects approximately 5-7% of racing Thoroughbreds worldwide resulting in debilitating muscle pain, cramps and, in severe cases, can result in euthanasia. The aetiology of recurrent exertional rhabdomyolysis however is poorly understood and therefore, the treatment of this disease is suboptimal. Symptomatic pain relief is the mainstay of treatment alongside husbandry changes to reduce the likelihood of recurrence.

There is some evidence that recurrent exertional rhabdomyolysis is associated with altered calcium regulation and vitamin D deficiency is also associated with altered calcium regulation in muscle cells. Vitamin D metabolism in horses remains poorly understood but it is thought that the main source of vitamin D comes from the horses' diet. Access to pasture provides a good source of dietary vitamin D and therefore, horses without grazing often have very low circulating vitamin D levels. Racing Thoroughbreds rarely have access to pasture and have been shown to have low vitamin D levels. However, the role of vitamin D in recurrent exertional rhabdomyolysis has not yet been explored.

This pilot study aims to evaluate vitamin D metabolism by comparing levels of vitamin D analytes in blood samples collected from 10 cases (recurrent exertional rhabdomyolysis affected horses) and 10 controls (unaffected horses). Blood samples will be collected at two timepoints; 1) within 24hours of an acute episode of recurrent exertional rhabdomyolysis and 2) 2-3 weeks after the acute episode to represent baseline levels. Each case will have a paired control horse from the same training yard and at a similar level of fitness to control for confounding variables such as gender and age. Blood samples will be analysed for total vitamin D status and other markers of altered vitamin D metabolism alongside markers of muscle injury. Husbandry and signalment data will also be collected for all horses included in the study.

The aim of this pilot study is to identify any differences in Vitamin D metabolism between case and control samples to support our hypothesis that vitamin D deficiency has a role to play in equine recurrent exertional rhabdomyolysis. This project could provide a vital step forward in understanding the aetiology of this debilitating disease and lead to improved treatment options.

### **Interdependence of structure and function: using electroanatomic mapping and MRI to determine the tissue characteristics driving propagation in the equine heart**

Principal Investigator: John Keen  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 30,732 - Pump-prime Funding 2024  
Project Period: December 2024 - November 2025  
Grant Reference No.: PPF-242016  
Status: **Active**  
Field of Research: Cardiovascular health and disease / Pathophysiology of cardiac and/or vascular disease / Identification of horses at increased risk of cardiovascular disease / Imaging

*Brief Summary: Arrhythmias in racehorses, especially atrial fibrillation, can lead to clinically significant health, performance, and safety (for riding/racing) problems. This project will further our understanding of the relationship between equine cardiac micro-structure and electrical function, offering the potential for horse-specific (personalized) investigations and treatments, thus improving veterinary care and clinical outcomes.*

Abnormal heart rhythms can lead to health problems in racehorses including: tiring during routine exercise, nasal bleeding, collapse, and even sudden death. The development of an abnormal rhythm depends on three main interdependent factors: the underlying tissue characteristics; the hormonal background that affects electrical activity; and the presence of abnormal 'early' heart beats that can act as a trigger for the rhythm disorder. A particularly common rhythm disorder in horses is atrial fibrillation (AF), where the top chamber of the heart fails to empty properly, leading to reduction in the heart's ability to pump as much blood as it would normally. This is more common in larger sports horse breeds, such as those involved in horseracing,

and thought to be due to their inherently large hearts and the high 'rest and digest' hormonal background. Interestingly AF also occurs in human patients and is the most common serious human heart rhythm abnormality worldwide. In both horses and humans, the precise mechanisms underlying development of AF in particular individuals, its persistence, and its recurrence following treatment are not understood.

We are aiming to develop new techniques for investigating and treating AF in horses, to understand why AF occurs in some horses and not in others and to also ensure that the techniques are safe and effective. These techniques (called 'mapping and ablation') are minimally invasive, using thin wires that are placed via veins into the heart that detect the cause of rhythm abnormalities and improve their treatment. These techniques are now used routinely in humans with heart rhythm problems including AF. In addition, we are using an advanced imaging technique called MRI to look at the detailed tissue structure in horses' hearts after death, so we can understand how this structure is related to the electrical signals in the heart. These studies will all be done in horses donated for euthanasia, where the owners have decided that this is the only humane option and when the owners want their horses contribute to advancing research into equine health and disease.

Ultimately this work will help us identify which horses are at risk of developing AF while also targeting new treatments to those at high risk of recurrence. In addition, due to the strong parallels with some aspects of human AF, it may also offer insight into this important condition in humans.

### **Investigation of cell free DNA as a biomarker of NETosis in airway lavage samples from horses with neutrophilic asthma**

Principal Investigator: Mary Sheats  
Administering Institution: North Carolina State University, US  
Amount Awarded: USD 38,507 - Pump-prime Funding 2022  
Project Period: July 2023 - December 2024  
Grant Reference No.: PPF-2022-100029  
Status: **Completed**  
Field of Research: Respiratory health and disease / Identification of risk factors associated with respiratory disease / Improved preventative and therapeutic strategies for lower airway disease in racehorses / Molecular biology / Inflammation / Immunopathology

#### **Plain Language Summary**

Our research investigated a novel biomarker for neutrophilic EA, with the goal of establishing this biomarker 1. as a diagnostic that can be measured on the farm, and 2. as a potential target for new therapeutic strategies for EA. The novel biomarker is cell-free DNA (cfDNA). Cell free DNA is known to be increased in airway samples from people with asthma because of increased release of neutrophil extracellular traps (NETs). Our study measured both NETs and cfDNA in airway samples from horses with mild/moderate equine asthma and severe equine asthma and compared them with healthy horses. We made these measurements with a small, portable, economical device that can be used stall-side on the farm to measure airway cfDNA. We determined that cfDNA is increased in the airways of horses with severe equine asthma but not mild/moderate equine asthma or healthy horse airways. We also used antibody-based testing to determine that the cfDNA in equine airways is coming from NETosis and not from dying or dead cells.

#### **Key Findings and Outcomes of the Project**

We hypothesized that cell-free DNA (cfDNA) would be increased in tracheal wash (TW) and/or bronchoalveolar lavage (BAL) fluid supernatant from horses with clinical signs and diagnosis of neutrophilic mild/moderate equine (N-mEA) asthma or severe equine asthma (sEA). Further, increased cfDNA in horses with neutrophilic equine asthma would be associated with an increase in neutrophil extracellular traps (NETs). We collected samples from 31 horses, both healthy university-owned horses and client-owned horses presenting for respiratory clinical signs. Horses received a physical exam with respiratory clinical scoring, upper airway endoscopy and mucus scoring, bronchoalveolar lavage (cytology, microscopy, cfDNA measurement, and measurement of citrullinated histone H3, cyclophilin A, caspase-cleaved K18, and myeloperoxidase.)

First, our team determined that cell-free DNA can be accurately measured with the small, portable Qubit 4 fluorometer and 1x dsDNA high sensitivity assay kit in both BAL and TW supernatant. cfDNA levels were highly correlated between the Qubit 4 fluorometer and Sytox plate-based fluorescence assay. Freeze/thawing of BAL/TW supernatant does not affect cell-free DNA measurement with the Qubit 4 fluorometer. Tracheal wash and bronchoalveolar lavage supernatant cfDNA is significantly higher in horses with sEA compared to horses with N-mEA, horses with non-neutrophilic asthma and healthy horses. Results from both the Qubit 4 fluorometer and Sytox plate-based assay demonstrate this difference.

Neutrophil extracellular traps (NETs) were identified via confocal microscopy in sEA BALF, staining for nuclear material, citrullinated histone and myeloperoxidase. NETs were not identified in N-mEA, non-neutrophilic EA, or healthy horse BALF.

ELISA results demonstrated significantly higher levels of myeloperoxidase and citrullinated histone H3 in sEA BALF supernatant compared to N-mEA, non-neutrophilic EA and healthy horse BALF supernatant. There was no difference in the levels of cyclophilin A or caspase-cleaved K18, demonstrating that the difference in cfDNA is due to NETosis vs. necrosis or apoptosis. CitH3 and MPO values were positively correlated with BALF % neutrophils. There was no correlation between cyclophilin A or caspase-cleaved K18 and BALF % neutrophils.

Completion of this research provides proof of principle evidence for cfDNA as a diagnostic biomarker of severe equine asthma, but not for neutrophilic mild/moderate equine asthma. The Qubit 4 fluorometer is a portable tool that may be useful as a stall-side device for measurement of cfDNA in equine airway lavage samples. The mechanism for increased airway cfDNA in horses with severe equine asthma is NETosis, and not apoptosis or necrosis.

### **Mapping the equine cardiac channelome – elucidation of molecular targets of electrophysiological function in horses with and without cardiac rhythm abnormalities.**

Principal Investigator: Rebecca Lewis  
Administering Institution: University of Surrey, UK  
Amount Awarded: GBP 179,036 - Major Research Grant 2021  
Project Period: October 2022 - December 2025  
Grant Reference No.: MRG-2021-101262  
Status: [Active](#)  
Field of Research: Cardiovascular health and disease / Molecular biology / Pathophysiology of cardiac and/or vascular disease / Physiology / Cardiac disease

#### **Publication:**

Charlotte E. Edling, Magdalena Arevalo-Turrubiarte, Antoine Premont, Marcos Castellanos Uribe, Bronte Forbes, Victoria Kemp, Joe Weir, Celia Marr, Rebecca Lewis, Kamalan Jeevaratnam, Gene expression patterns of the four cardiac chambers in the Thoroughbred horse, *Journal of Equine Veterinary Science*, Volume 149, 2025, 105415, ISSN 0737-0806, <https://doi.org/10.1016/j.jevs.2025.105415>.

Horses have a unique ability to dramatically alter their heart rate to cope with increased energy demand, with 7-8 fold increases at peak exercise performance. Such increases in heart rate are unattainable in other mammals, where they would be considered damaging, with a very high likelihood of mortality. The electrical excitability of the heart plays an important role in modulating its activity and in providing this unique capacity to cope with strenuous exercise. However, the mechanisms by which electrical activity is controlled are unknown in the horse.

Each year horses die on the racetrack, leading to public outcry and negative publicity for the equine racing industry. Irregular heart rhythms are an important cause of sudden cardiac death (SCD) in horses. In humans, these abnormal rhythms are typically associated with alterations in ion channels (particularly their number and location within the heart), which conduct electrical activity across the heart. Human investigations have focused on understanding these ion channels in healthy humans and humans with abnormal heart rhythms, in order to produce novel and more effective drugs. However, this important knowledge is not available to equine veterinarians and researchers.

Here we will use advanced molecular methods to study ion channels and how they are distributed across the entire equine heart and compare hearts from horses without abnormal rhythms to those that have abnormal heart rhythms. We will then correlate this with the different ways the proteins are made, modified and regulated in the different areas of the heart to determine how the path of conduction is altered to cause the rhythm abnormality.

This will enable us to identify the specific changes which occur in those genes and proteins responsible for cardiac conduction in horses and determine how this influences the function of the heart. This will vastly increase our knowledge of these critically important channels, and will lead to improved ability to diagnose, treat, and prevent potentially fatal dysrhythmias in horses. The ultimate aim of the research will be to fundamentally better understand how the horses' heart functions allowing for better management, prevention and treatment of disease and conditions related to the equine heart, particularly those linked to SCD. Given the importance of reducing the rate of SCD for the equine industry, this study represents a critical first step to understanding why certain horses develop dysrhythmias. Ultimately, this proposal is a key first step to reducing the rate of SCD in equine athletes.

### **Prediction and prophylaxis of EIPH during racing by on-board monitoring of horses during training - a pilot study**

Lead Applicant: Emmanuelle Van Erck-Westergren  
Host Organisation: Equine Sports Medicine Practice, Belgium  
Amount Awarded: EUR 13,488 - Small Research Project 2021  
Project Period: February 2022 - January 2023  
Grant Reference No.: SRP-2021-101756  
Status: **Completed**  
Field of Research: Respiratory health and disease / Improved diagnosis for lower airway disease / Exercise-induced Pulmonary Hemorrhage / Sports medicine

#### **Plain Language Summary**

The Hong Kong EIPH (Exercise-Induced Pulmonary Hemorrhage) pilot study aimed to investigate if EIPH could be predicted and prevented in racehorses through on-board monitoring of several of the horses' fitness parameters during training. The researchers are seeking to understand the pattern abnormalities associated with EIPH during intense exercise and develop methods for early detection and prediction. They utilized the Equimetre monitoring system and conducted a pilot study during barrier trials in Hong Kong.

The objectives of the pilot study were to demonstrate collecting fitness and locomotor parameters was feasible during intense exercise with the Equimetre device and harvest simultaneous clinical data post-exercise by performing endoscopic examinations to detect and quantify EIPH of varying severity. This data is essential to record on a larger number of horses in a larger study to determine the predictive value of these abnormalities measured during training for EIPH during racing.

The Equimetre device, attached to the horses' girth, recorded various variables such as heart rate, ECG, speed, stride length, and gait symmetry. Post-exercise, unsedated horses underwent endoscopic examinations to grade the severity of EIPH on a scale of 0 to 4. The researchers analyzed ECG recordings for the presence of arrhythmias.

The results indicated that the study was feasible. Fifty-six horses participated in barrier trials, and data on speed, gait, and physiological variables were successfully collected. Out of 51 ECG recordings analyzed, arrhythmias were present during exercise in 37 horses, with 28 horses showing arrhythmias at maximal exercise. Isolated premature complexes were observed, with some horses having multiple arrhythmias. Atrial fibrillation was not detected.

The association between arrhythmias and EIPH severity was examined. The data showed varying proportions of arrhythmias among different grades of EIPH. The researchers will further investigate the association between EIPH and variables such as arrhythmias, peak speed, stride frequency, stride length, and heart rate recovery.

Overall, the pilot study demonstrated that the Equimetre system effectively monitored racehorses during maximal exercise in barrier trials, and video endoscopy after exercise was feasible. The detection of arrhythmias during high-intensity exercise highlighted their presence in Hong Kong racehorses. The results provided assurance that a larger, definitive study is achievable and necessary.

This pilot study confirmed the feasibility of the research and provided valuable baseline data for designing a larger, definitive study. The results indicated the presence of arrhythmias during exercise and the potential association between arrhythmias and EIPH severity. The ultimate goal is to develop effective methods for predicting and preventing severe EIPH in racehorses, improving their performance and well-being.

### Key Findings and Outcomes of the Project

The results should be considered in light of the purpose of this pilot study, which was to address the question – can this study be done. This can be addressed by considering:

**Management:** The results indicate that the study is achievable in that exploitable 50 records were made of 56 horses indicating that horses can be recruited to the study, instrumented before the barrier trial, and examined within an appropriate time frame after the end of exercise. That some horses were examined more than once indicates a willingness by some trainers to have horses examined. Management questions that remain for a definitive study include:

1. Can a sufficient number of horses be recruited to provide valid results (number based on a power analysis)?
2. Are there sufficient personnel resources in Hong Kong to complete a larger study?
3. How long would it take to recruit a sufficient number of horses given available personnel and facilities resources?

**Technology:** We have demonstrated that the Equimetre system can be applied to horses in a barrier trial, data on a number of variables collected and exported for analysis, and video-endoscopy performed and recorded with diagnostic quality. Importantly, the pilot trial revealed that this data can be transmitted electronically globally. Technological questions that remain are:

1. Is there availability of a sufficient number of Equimetre devices to allow efficient conduct of the study?
2. Does the video endoscopy and recording equipment need to be upgraded?
3. Is the ECG data analysis dependent on particular software? Should this be purchased or upgraded?

**Resourcing:** Resourcing was adequate for the pilot trial. The trial provided an indication of the extent of resourcing needed to complete the definitive study including:

1. What personnel resourcing is available to oversee barrier trials, identify and recruit horses, instrument horses, perform video-endoscopy and undertake record keeping required to enroll horses?
2. Are there sufficient Equimetre units available for the definitive study?
3. Is there sufficient capacity to provide analysis of the ECG and video-endoscopy data?

**Horses:** The pilot study provided baseline data that enables a series of power calculations to determine the minimum number of horses need to complete the study to provide a statistically valid assessment of key outcome variables. Calculation of the number of horses needed for the definitive study will include a drop out rate of 10% (five of 56 ECG's were uninterpretable), based on the pilot study, meaning that the final number of horses enrolled will be determined by the number needed for a valid statistical analysis taking into account that 10% of enrolled horses will either not complete the study or have faulty recordings.

**Detailed results:** The pilot trial yielded the following results, which will be used to design the definitive trial. A total of 52 horses were examined during barrier trials with 4 horses examined more than once for a total of 56 recordings. Comprehensive speed and gait data were obtained for all horses. Fifty-six ECGs were evaluated. Five ECGs were excluded from analysis due to excess motion. In the remaining 51 ECGs, the average maximal heart rate was 216 bpm. Arrhythmia was present at any portion of exercise in 37 horses. At maximal exercise, arrhythmia was present in 28 horses, with the number of isolated premature complexes at maximal exercise ranging between 1 and 17. Of these, 14 horses had 3 or more isolated arrhythmias at maximal exercise and 7 horses had 5 or more isolated arrhythmias at maximal exercise. When considering horses with arrhythmia in any

portion of exercise, 15 horses had APCs, 20 horses had VPCs and 2 horses had both APCs and VPCs. The arrhythmias were isolated in most horses, with couplets found in 5 horses (all of these couplets were during submaximal exercise (n=1) or in the recovery phase (n=4)). Atrial fibrillation was not detected in any of the horses. A majority of horses had an episode of EIPH with a proportion of horses having an EIPH score >1 (34/47 (72%) vs. horses with no evidence of blood post trial (13/47 (28%)). The proportion of horses with an arrhythmia during exercise and an EIPH grade >1 was 77% (27/35), indicating that the association between arrhythmia and EIPH warrants further investigation. Variables examined for any association (odds ratio determined by multiple logistic regression) with EIPH (Y/N, EIPH > 1, EIPH > 2, EIPH >3) will include:

- Presence of arrhythmia during maximal exercise
- Presence of atrial fibrillation
- Presence of < 5, 5-10, or >10 ectopic beats during maximal exercise
- Peak speed
- Time to perform fastest 200m
- Time to perform fastest 500m
- Peak stride frequency
- Peak stride length
- Stride frequency at 50 km/h
- Heart rate after fast recovery
- Heart rate after 15 min recovery
- Heart rate after 15 min recovery in percent of maximum heart rate

#### Discussion

This pilot study undertaken at the HKJC racetracks shows that the comprehensive monitoring of racehorses during maximal exercise at Barrier Trials was reliable using the Equimetre system and that video trachea-bronchoscopic examination of horses after exercise was feasible through the collaboration with the local veterinary team lead by Dr O'Connor. Arrhythmia detection is feasible in the Equimetre ECGs at high intensity exercise. Arrhythmias are present during exercise in horses in Hong Kong. Further analysis to determine their importance of these findings relies on examination of a larger number of horses. The results of this pilot study provide assurance that a larger, definitive study is achievable and warranted.

### **The effects of short-term omeprazole on serum gastrin and chromogranin A, as markers of rebound gastric hyperacidity, in the horse.**

Principal Investigator: Benjamin Sykes  
Administering Institution: Massey University, New Zealand  
Amount Awarded: NZD 33,235 - Pump-prime Funding 2021  
Project Period: January 2022 - December 2022  
Grant Reference No.: PPF-2021-102068  
Status: **Completed**  
Field of Research: Gastrointestinal health / Equine Gastric Ulcer Syndrome / Immunology / Gastroenterology / Toxicology

#### Publications:

- ▶ Clark B, Steel C, Vokes J, et al. Evaluation of the effects of medium-term (57-day) omeprazole administration and of omeprazole discontinuation on serum gastrin and serum chromogranin A concentrations in the horse. *J Vet Intern Med.* 2023; 37(4): 1537-1543. <https://doi.org/10.1111/jvim.16795>
- ▶ Vokes, J.R.; Gedye, K.R.; Lovett, A.L.; de Kantzow, M.C.; Shan, R.; Steel, C.M.; Sykes, B.W. Evaluation of Two Commercial ELISA Kits for Measuring Equine Serum Gastrin Compared to Radioimmunoassay. *Animals* 2024, 14, 2937. <https://doi.org/10.3390/ani14202937>

#### **Plain Language Summary**

Rebound gastric hyperacidity (RGH) secondary to hypergastrinemia has been suggested to contribute to the rapid recurrence of equine squamous gastric disease following the discontinuation of omeprazole. The aim of

the study was to evaluate changes in serum gastrin and chromogranin A (CgA) concentrations in response to medium-term (57-day) omeprazole treatment and following omeprazole discontinuation.

Fourteen mature Thoroughbred racehorses in simulated race training were enrolled in the study. Horses received 2.28 grams of oral omeprazole once daily for a total of 57 days within a 61-day period, excluding a withholding period applied mid-protocol where treatment was stopped as part of a concurrent study. Serum samples were collected on day 0 prior to omeprazole treatment, on day 1 of each week of the treatment period, and for a further five weeks post-discontinuation of treatment. Serum gastrin and CgA concentrations were analyzed using radioimmunoassay (RIA) analysis and ELISA, respectively.

Median serum gastrin concentrations had a 2.5-fold increase from baseline to day 7 ( $p < .001$ ) but did not increase further during the omeprazole treatment period. Median serum gastrin concentrations returned to baseline within 2-4 days after administration of the last dose of omeprazole. No effect of treatment or discontinuation was seen in serum CgA concentrations. The results of the current study do not support the use of tapering protocols in horses.

### **Unravelling the toxic and pro-inflammatory potential of inhalable mineral dusts generated from racehorse working surfaces**

Principal Investigator: Michela Bullone  
Administering Institution: University of Turin, Italy  
Amount Awarded: EUR 32,500 - Pump-prime Funding 2023  
Project Period: April 2024 - April 2025  
Grant Reference No.: PPF-2023-232005  
Status: [Active](#)  
Field of Research: Respiratory health and disease / Identification of risk factors associated with respiratory disease / Prevention of lower airway disease / Inflammation / Equine welfare

*Brief Summary: The proposed project will inform, for the first time, on the toxic and pro-inflammatory potential of footing surfaces commonly employed in the equine racing industry, ultimately contributing to the development of management practices aimed at improving the respiratory health and welfare of racehorses while training and racing.*

The second leading causes of reduced performance in racehorses, after musculoskeletal injuries, is represented by respiratory problems. Most of these are inflammatory in origin, meaning that the horse respiratory system reacts to external stimuli that can be considered dangerous to for the equine respiratory system. This stimuli are many and can be represented by infectious agents, such as bacteria and or viruses, by inhaled organic compounds (such as pollens, mold and fungal wall particles) or by inorganic irritants (such as ammonia, for example). When these compounds are present in high quantity within the air, air quality is reduced and the risk of lung disease may increase.

Among air contaminants with high toxic potential for the airways, there is silica dust, also known as RCS (respirable crystalline silica). Silica is highly abundant on Earth as it composes many sands and rocks, and we all are exposed to silica daily. However, silica becomes particularly aggressive for our respiratory system when its particles are so small they can reach the most distant part of the lung (the alveoli) and when it is freshly fractured. Most equine surfaces are composed of silica in variable parts (40-90% reported in North America). The friction imposed by equine hoofs on such surfaces, especially at high speeds, can generate surface dusts of such a small size they can reach the lungs and produce an inflammatory response. Data on this regard are currently lacking in horses, however.

With the current proposal, we would like to study the composition and toxic and inflammatory potential of a representative sample of 15 currently employed surfaces in racing and training facilities for racehorses in our region, and to relate these data with their RCS content.

To do so, we will characterize the particle size distribution and mineralogical composition of the collected surfaces, we will treat them to inactivate non-mineral components and we will use chemical and biological tools to determine the outcomes of interest. A special machine will determine the free radical (oxidant)

generation capacity of the surface studied and their dusts. Other tests conducted in laboratory will assess the interaction of the surfaces and their dusts on equine red blood cell membrane. If the red blood cells rupture, it indicates a high toxic potential of the compound tested. Finally, cells harvested immediately postmortem from equine lungs will be cultured in the presence or absence of different concentrations of the surfaces and their dusts, and their production of pro-inflammatory mediators will be monitored in time. Altogether, these data, after appropriate statistical analysis, will provide answers to our research questions and will determine whether more research efforts will have to be granted on this subject. In particular, the next step would be a large-scale clinical study to determine whether a link exists between the prevalence of equine respiratory diseases in racehorses and surface-generated dust exposure and to establish guidelines (on surface choice and/or management practices) for safe breathing in equine racing environments.

## Husbandry and management

### Automatic behaviour recognition using wearable sensors for improving horse health and welfare

Principal Investigator:	Kai Liu
Administering Institution:	City University of Hong Kong, HKSAR, China
Amount Awarded:	HKD 294,987 - Pump-prime Funding 2022
Project Period:	June 2023 - May 2024
Grant Reference No.:	PPF-2022-100030
Status:	Completed
Field of Research:	Well-being of the racehorse / Tools to quantify a horse's welfare status / Retirement and careers beyond racing / Equine behaviour / Wearable device / Equine welfare / Retirement from racing

#### Plain Language Summary

Our project aims to enhance horse welfare and health by monitoring and understanding their behaviour, which is crucial for ensuring their overall well-being. Traditionally, observing horse behaviour has depended heavily on expert assessments. Although these professionals are highly skillful, their work is often time-consuming, labor-intensive, and susceptible to personal biases. To address these challenges, we have explored the integration of advanced technology, specifically by combining sensors with machine learning. This approach creates a more accurate and objective system for tracking and analyzing horse behaviour, overcoming the limitations of traditional methods.

We have developed a system that uses deep learning to monitor the behaviour of retired racehorses in real time. We fitted racehorses with small, wearable sensors that utilize inertial measurement units (IMUs) to measure motion via accelerometers and gyroscopes. These sensors were positioned on the horses' necks to record their movements during daily activities such as eating, standing, shaking, and walking. Over two days, we collected a substantial dataset of more than 50 million data points at a frequency of 52 times per second. This data was meticulously labeled using a specialized tool we developed, which synchronized the sensor data with video footage to accurately identify the observed behaviours.

The core of our approach was the deployment of a sophisticated machine learning model called CMIM-Net. This model is specifically designed to analyse the complex data captured by the sensors. It processes the data through two distinct pathways, each dedicated to a different type of movement data—one for acceleration and one for rotational movement. Utilizing a technique known as the Cross-Modality Interaction Module, our model intelligently integrates and analyses this data, focusing on the most relevant information while filtering out noise and irrelevant signals. This approach significantly enhanced the model's ability to accurately recognize various behaviours, even in the presence of imbalanced data, where some behaviours, such as shaking or walking, occurred less frequently than others.

During the training process, we encountered the challenge of an uneven distribution of behaviours in our dataset. To address this issue, we employed a technique known as Class-Balanced (CB) focal loss, which directs the model's focus toward less common or harder-to-recognize behaviours. This technique was essential for ensuring that our model could accurately detect all behaviours, not just the most frequent ones. Following the training, our model achieved an impressive accuracy rate of 87.46%, demonstrating its reliability and effectiveness.

We went beyond just developing the model by implementing a real-time system. The data collected by the sensors is transmitted to a cloud server, where our deep learning model processes it almost instantly. The results are then relayed back to a mobile device, enabling caretakers to monitor the horse's behaviour in real-time. This system not only alleviates the workload for veterinarians and caretakers but also offers a scalable solution suitable for various settings, thereby enhancing the management of horse health and welfare.

In summary, our work marks a significant advancement in utilizing technology to monitor horse behaviour. By integrating advanced machine learning techniques with real-time data processing, we have developed a

system that is both efficient and effective in tracking the health and well-being of horses. This innovation has the potential to revolutionize horse care by enabling earlier detection of health issues and improving overall welfare management.

### Key Findings and Outcomes of the Project

The behaviour of horses is a vital indicator of their mental and physical well-being, reflecting their overall health and welfare. Traditionally, monitoring horse behaviour has depended on observations by trained professionals, a method that is not only time-consuming and labour-intensive but also prone to subjective bias. Integrating sensors with machine learning technology offers a more precise and objective approach for behaviour recognition and monitoring. This advanced method facilitates real-time detection of behavioural changes, thereby significantly alleviating the workload of veterinarians and caretakers. Moreover, it enables early identification of potential health issues, allowing for timely interventions that improve the care and management of horses.

The key findings and outcomes of our project include the successful implementation of a deep learning-based approach that leverages a Cross-Modality Interaction Network to recognize and predict the real-time behaviours of racehorses in stables, achieving an accuracy of 87.46%. By collecting and labeling Inertial Measurement Unit (IMU) data for four specific actions — eating, shaking, standing, and walking — from ten retired racehorses in stables, we developed a model capable of tracking behaviours on a per-second basis. This data is analyzed in real-time on a cloud server and transmitted the result of racehorse behaviour to a mobile device via Bluetooth, offering an effective solution for monitoring the health and welfare of horses. The detailed findings and outcomes can be summarized as follows.

1. **Dataset Construction Phase:** Our project successfully involved the collection and precise recording of over 50 million IMU (Accelerometer and Gyroscope) data points. These data were gathered using Movesense sensors attached to the necks of ten racehorses at the Hong Kong Jockey Club's Sha Tin base. The sensors recorded data at a frequency of 52 Hz over approximately 65.5 hours, spanning two different days. The collected data were categorized into four specific behaviors: standing (40%), eating (58%), shaking (1%), and walking (1%), reflecting an imbalance in the dataset. To address the challenge of accurately labeling behaviours based on sensor data alone, we developed an enhanced labeling tool using MATLAB. This tool synchronized video footage with the corresponding sensor data, enabling precise annotation of behaviours. This meticulous labeling process was essential for training the neural network models used in the subsequent behaviour recognition phase.
2. **Model Construction Phase:** We employed a cutting-edge Cross-Modality Interaction Network (CMI-Net) to significantly enhance the accuracy of recognizing horse activities using accelerometer and gyroscope data. The CMI-Net architecture features a dual Convolutional Neural Network (CNN) trunk, incorporating a residual-like convolution block (Res-LCB). This block is pivotal for improving the model's representation ability and overall robustness, allowing it to effectively process complex data inputs. A key innovation in this approach is the integration of the Cross-Modality Interaction Module (CMIM), which utilizes an advanced attention mechanism. The CMIM captures complementary information from multi-modal sensor data while actively suppressing irrelevant signals, such as noise and redundant or confusing inputs. This capability enables the model to concentrate on the most pertinent features, resulting in more accurate behaviour recognition. Furthermore, the CMIM introduces a novel attention module that facilitates deep intramodality interaction by combining spatial information from two-stream feature maps through basic CNN. This process generates spatial attention maps that adaptively recalibrate temporal and axis-wise features within each modality, thereby refining the model's predictive power. It is noteworthy that applying attention mechanisms in horse activity recognition, especially using multi-modal data from wearable sensors, represents a novel approach in the field. To address the challenge posed by the imbalanced dataset—characterized by uneven distribution of the four classified behaviours—we employed a Class-Balanced (CB) focal loss function during CMIM-Net training. This loss function is crucial for ensuring that minority class samples, which occur less frequently, receive adequate attention during model optimization. Additionally, the CB focal loss emphasizes samples that are difficult to classify, preventing them from being overlooked in the learning process. To our knowledge,

this is the first application of CB focal loss in horse activity recognition for solving imbalance datasets, highlighting the innovation and effectiveness of this approach.

3. **Model Training Phase and Results:** Our program demonstrates that the CMIM-Net model achieved an accuracy of 87.46% and an F1 score of 0.84. When evaluated on data from two additional horses, the model's accuracy for the four behaviours was as follows: 88.38% for standing, 86.15% for eating, 82.51% for shaking, and 84.09% for walking. During model training, the dataset was partitioned into a 3:2:2 ratio for each class label, with 52 IMU data points forming a segment fed into the CMIM-Net. The network was designed with two distinct CNN branches—CNNacc for accelerometer data and CNNgyr for gyroscope data—to extract modality-specific features. These features were concatenated before being processed by the final dense layer. To ensure effective interaction between the two modalities and to capture complementary information while filtering out irrelevant data, a joint Cross-Modality Interaction Module (CMIM) was integrated into the upper layer of the network. This integration contributed to achieving the model's optimal performance.
4. Our project highlights the successful integration of cloud-edge fusion technology for real-time horse behaviour recognition. We deployed the CMIM-Net model on a cloud server, creating an innovative system that enables data transmission. Sensor data from Movesense devices, worn by the horses, is real-time transmitted via Bluetooth to a mobile device. This mobile device automatically uploads the IMU data to the cloud server, where the deep learning model processes and analyzes it. The analyzed results are then promptly returned to the mobile device, facilitating real-time behaviour recognition and display. This approach not only improves the accuracy and efficiency of monitoring horse behaviours but also offers a scalable solution for real-time health and welfare management in horse care.

In summary, our project offers a modern and efficient method for monitoring four key horse behaviours, which can significantly enhance the care and management of horses. This innovation has the potential to revolutionize how we observe and manage horse health, leading to improved health outcomes and overall welfare.

### **Bugs, Bones and Vitamin D - A pilot study: Developing novel tools to assess bone health and reduce the risk of bone fractures.**

Principal Investigator:	Chris Proudman
Administering Institution:	University of Surrey, UK
Amount Awarded:	GBP 19,223 - Pump-prime Funding 2023
Project Period:	November 2023 - October 2024
Grant Reference No.:	PPF-2023-232003
Status:	Completed
Field of Research:	Musculoskeletal injury and disease / Strategies to prevent musculoskeletal disease and injury / Orthopaedic disease / Metabolism / Microbiology

#### **Plain Language Summary**

This pilot study was designed to develop and to test some of the research tools required to conduct a larger study exploring the relationship between gut bacterial communities, blood vitamin D status, calcium metabolism and bone strength in horses. The ultimate aim of this work is to develop novel approaches to healthy bone development in foals that will decrease the risk of fractures when racing. Important and relevant knowledge and experience were achieved under each study objective which have been carried forward into the design of a larger, definitive study.

#### **Objective 1: Measure vitamin D and related metabolites in the blood of six Thoroughbred mares and their foals over a 6-month period.**

This objective was achieved through collaboration with the quality assured laboratory at East Anglia Bioanalytical Facility, Norwich. Our results indicate rapidly rising blood concentrations of vitamin D in foals between birth and six months of age. Importantly, concentrations of vitamin D measured are significantly lower than those found in humans.

**Objective 2: Quantification of dietary intake of calcium and vitamin D by the six mares on the study.**

A protocol for feed sampling and testing was created and used to generate an accurate understanding of vitamin D and calcium intake by the six mares and foals in this pilot project. Surprisingly, we detected significant discrepancies between manufacturer-declared vitamin D<sub>3</sub> content of some feedstuffs and independent laboratory measurement. We used these measurements to calculate the daily dietary intake of vitamin D in foals. On average, foals were receiving only 80% of the recommended intake.

**Objective 3: Measure bone strength in six Thoroughbred mares and their foals over a 6-month period.**

We purchased a novel piece of equipment that measures, non-invasively, the speed at which sound travels through bone. This has been validated as a measurement that is highly correlated with bone strength. We developed protocols for taking these measures in the mares and foals on the study and demonstrated a high level of agreement between repeated measurements at the same site. We documented the average speed of sound measurements from three different sites on the same bone in our six study foals and tracked the development of bone strength (measured as speed of sound) up to six months of age.

Summary: This pilot study has allowed us to successfully develop methods to measure vitamin D in the blood of foals, measure vitamin D intake in a foal's diet and measure bone strength using a non-invasive, speed of sound technique. This work has been instrumental in allowing the project team to develop a full application which was submitted to the Foundation for consideration in February 2025.

**Key Findings and Outcomes of the Project**

This pilot study tested the feasibility and reliability of some of the research tools required to conduct a full-scale study exploring the relationship between gut bacterial communities, vitamin D status, calcium metabolism and bone strength in foals.

**Hypothesis:** We hypothesised that we could reliably and repeatably measure serum vitamin D status, markers of calcium metabolism and bone strength in foals and adult horses.

**Objective 1:** Measure serum levels of i) 25-hydroxyvitamin D, ii) 1,25-dihydroxyvitamin D, iii) vitamin D binding protein, iv) parathyroid hormone, v) calcium, and vi) albumin in six Thoroughbred mares and their foals over a 6-month period.

**Outcomes:** We successfully acquired excess blood sample from 6 mares and from foals at 1, 10, 90 and 150 days of age. Samples from mares were acquired before and six weeks after daily supplementation of feed with a commercial vitamin D<sub>3</sub> supplement. Laboratory analysis was undertaken by the University of East Anglia Bioanalytical Facility, Norwich, which participates in international quality assurance schemes.

Selected analyte concentrations that are important to calcium, vitamin D and bone metabolism are illustrated in Figure 1. Serum total 25(OH) vitamin D concentrations for all six foals demonstrated a rapid increase in concentration with age during the first five months of life (Figure 1A). The vitamin D concentrations observed were below the threshold of rickets/osteomalacia definition in humans (~ 5-10nmol/l for total 25OHD). The ratio of total 25(OH) vitamin D: total 24, 25(OH)<sub>2</sub> vitamin D (Figure 1B) for our paediatric foal population is markedly lower than the ratios observed in humans (10:1), indicating enhanced conversion of 25(OH) vitamin D to 24, 25 (OH)<sub>2</sub> vitamin D in horses.

Our data indicate increasing blood calcium concentration and albumin concentration with age (Figures 1C and 1D). Figures 1E and 1F arise from opportunistic sampling of the six study mares before and after dietary supplementation with vitamin D. The graphs show an increase in serum total 25(OH) vitamin D after supplementation (Figure 1E) which is largely due to an increase in total 25(OH) vitamin D<sub>3</sub> which is rapidly absorbed from the GI tract.

Four samples were assayed for parathyroid hormone, which was expensive, and we were unable to source a vitamin D binding protein measurement service.

**Objective 2:** Quantification of dietary intake of calcium and vitamin D by the six mares on the study.

**Outcomes:** A protocol for feed sampling and testing was created and used to generate an accurate understanding of vitamin D intake by the six mares and foals in this pilot project. Results of feed analysis by an independent, quality assured laboratory are indicated significant discrepancies between manufacturer-declared vitamin D3 content and laboratory measurement. Two of the sampled feedstuffs contained <50% of the manufacturer’s declared vitamin D3 concentration.

Using these data, foal/mare bodyweight and quantity of each feedstuff given to each animal, we were able to estimate average daily vitamin D intake for pre-foaling mares, post-foaling mares and weaned foals (Table 1). These figures were compared against NRC recommended minimum requirements. On average, foals were receiving dietary vitamin D below the recommended value.

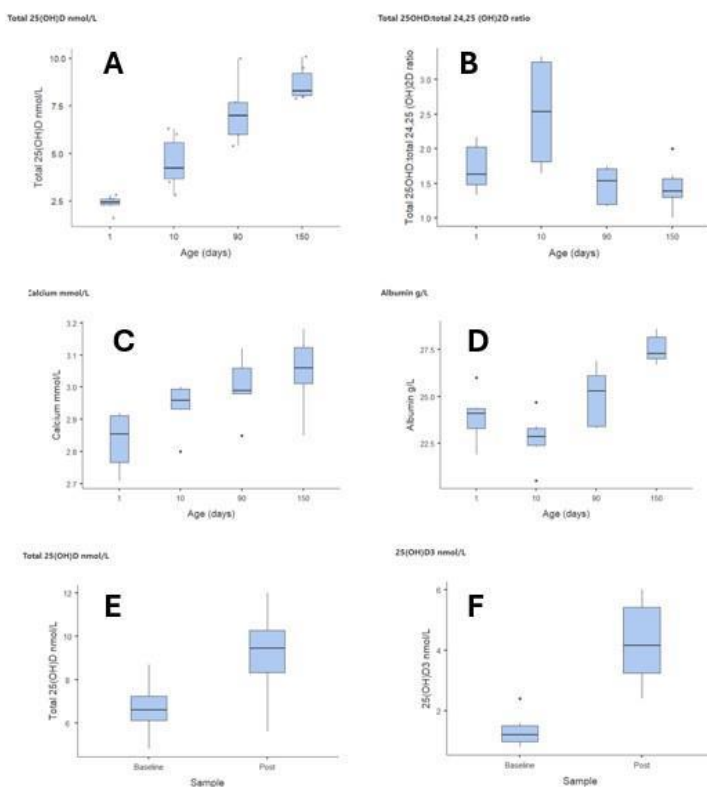


Figure 1: Serum concentration of analytes associated with vitamin D and calcium metabolism in a study population of 6 Thoroughbred mares and their foals. A) Total 25(OH) vitamin D in foals aged 1 – 150 days old; B) 25(OH) vitamin D: 24,25 (OH)2 vitamin D ratio for foals aged 1 – 150 days old; C) and D) serum calcium and albumin concentration (respectively) for foals aged 1 – 150 days old; E) Total serum 25(OH) vitamin D before (baseline) and six weeks after (Post) supplementation with Cal+; F) Total serum 25(OH) vitamin D3 before (baseline) and six weeks after (Post) supplementation with Cal+.

Table 1. Daily vitamin D (IU/day) intake based on lab analysis vs NRC (2007) minimum requirement recommendations.

	Requirements (minimum NRC 2007)	Intakes iu Vit D /day	Differences iu Vit D	% requirements
Pre-foal mares	5100	12000	7200	240
Post-foal mares	5100	35000	30000	680
Weaned foals	6800	5400	-1500	79

**Objective 3:** Measure bone strength using ultrasonic speed of sound in six Thoroughbred mares and their foals over a 6-month period.

**Outcomes:** Purchase of a Beam Med Omnisense 8000 ultrasound machine was completed in January 2024 and the research team spent time developing protocols for use. Measurement acquisition time was minimized to facilitate measurements in foals that were minimally restrained and initial problems with skin contact were overcome by the pre-application of ultrasound coupling gel. Measurements of ultrasonic speed of sound (SoS) through bone at three locations on the mid-metacarpus were recorded, dorsal, lateral and medial. Consolidated SoS data from the mares, taken at two different time points, was used to estimate the coefficient of variation in measurements (Table 2). These data indicate a coefficient of variation below 5% which is a widely accepted benchmark for repeatability.

Table 2. Descriptive data for SoS measurements in mares (n = 8 measurements for each location), taken at two different time points.

	Mean	Standard deviation	Coefficient of variation (%)
Dorsal	3758	157	4.17
Lateral	4317	173	4.00
Medial	4065	130	3.19

SoS data were acquired from our six study mares to establish baseline values for SoS at different anatomical locations (Figure 2). In each horse, the left foreleg metacarpal III was used to acquire SoS measurements from lateral, dorsal and medial mid-metacarpus. The pattern of SoS measurements is similar to that reported in cadaver limbs (Carstanjen et al., 2003) with lateral and medial metacarpus locations consistently returning higher SoS values than dorsal metacarpus.

SoS data from foals (n = 6) indicates a small range of values across the different leg locations at one month old, with differentiation towards the adult pattern of values by six months old (Figure 3). The difference between SoS values for the dorsal McIII vs McIII at other locations is statistically significant (ANOVA P<0.001).

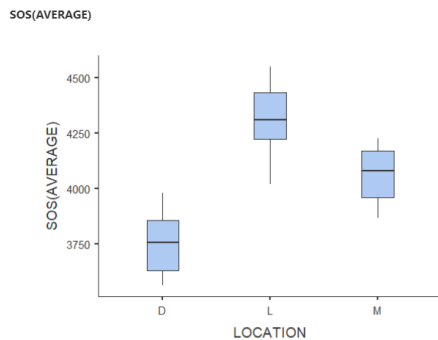


Figure 2. Mean SoS measurements for mares (n =6) at different leg locations.

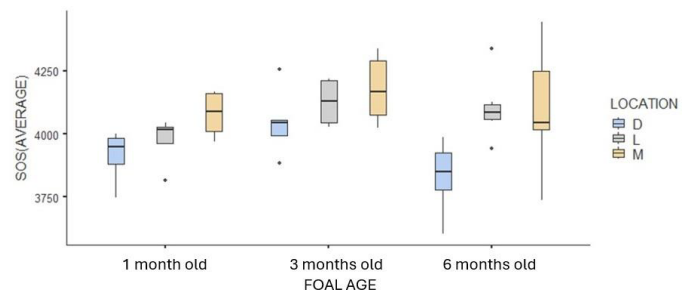


Figure 3. Foal SoS data, at each leg location, grouped by age at acquisition.

## Developing novel tools towards the reduction of antibiotic usage and against antimicrobial resistance in equine infection, by using stem cell approaches

Principal Investigator: Cristina Esteves  
 Administering Institution: The University of Edinburgh, UK  
 Amount Awarded: GBP 30,261 - Pump-prime Funding 2023  
 Project Period: April 2024 - January 2025  
 Grant Reference No.: PPF-2023-232008  
 Status: **Completed**  
 Field of Research: Infectious disease / Control and prevention of current and emerging infectious diseases / Treatment of current and emerging infectious diseases / Molecular biology / Antimicrobial resistance / Microbiology

### Plain Language Summary

The development of antibiotic-resistant bacteria is a growing problem to animal and human health worldwide. Treatment of equine infections, particularly those caused by antibiotic-resistant bacteria, can be challenging, detrimental to patient welfare, and costly. Such treatments are often associated with low performance and lost training days, particularly in racing thoroughbreds. Therefore, there is a pressing need to develop new and clinically effective ways to manage infections whilst also reducing antibiotic use to slow the emergence of more antibiotic-resistant bacteria strains.

One potential novel method to combat equine infections is the use of mesenchymal stem cells (MSCs). These MSCs are already used for equine musculoskeletal repair, where they promote tissue healing. However, they have also been shown in humans and dogs to secrete products into their environment that can promote bacteria death either by acting directly on the bacteria or by enhancing the activity of the immune cells.

In this project we investigated the effects of the secreted products from MSCs either previously collected from horses or generated in the lab (induced MSCs – iMSCs). We applied the secreted MSC products to bacteria isolated from horses which had some resistance to antibiotics. This allowed us to determine the effect of these products on bacterial growth and whether they interacted with the addition of antibiotics.

We found that MSC-secreted products inhibited the growth of bacteria species *Staphylococcus aureus* and *Streptococcus equi zooepidemicus*, but had no effect on the growth of *Escherichia coli*. iMSCs acted similarly to MSCs in all conditions and therefore constitute an advantageous alternative cell source to the classical cells obtained from animals. Addition of sublethal levels of antibiotics had no additional effect when combined with the MSC-secreted products, suggesting that the secreted products may not enhance the bactericidal effect of low levels of antibiotics. These assays need further optimization, as we have observed positive effects in other species.

These findings suggest that MSC-secreted products could in the future be used as alternative or in combination with antibiotics to treat equine infections, especially those produced by bacteria resistant to antibiotics.

### Key Findings and Outcomes of the Project

In this project we aimed to investigate the activity of equine conditioned media (CM) collected from mesenchymal stem cells (MSCs) against multi-drug resistant (MDR) pathogenic bacteria isolated from equine clinical specimens. Clinical isolates often act differently to commonly-used lab strains, therefore understanding how they respond to treatments is of great interest. Treatment of equine infections, particularly those caused by antibiotic-resistant bacteria, can be challenging, detrimental to patient welfare, and costly. Such treatments are often associated with low performance and lost training days, particularly in racing thoroughbreds.

Initial experiments were performed to optimise the growth and subsequent analysis of the bacteria isolates. Three bacteria species were investigated – *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus equi*, species which are commonly found to be MDR in equine clinical isolates. Prior to the start of the project a selection of strains of these bacteria species were characterised in terms of their susceptibility or resistance to a range of commonly used antibiotics. Strains were selected for further investigation based on their extent of growth in a CLARIOstar machine. It was found that, with some optimisation, all three species could be consistently grown in a CLARIOstar machine with both mammalian cell and bacterial cell media. They could also be consistently grown on agar plates for measurements of CFUs.

More optimisation experiments were required to ensure appropriate collection of MSC-CM. A range of cells were used to compare their effects. NBL6, an equine fibroblast cell line was grown to act as control. Endometrium MSCs (EM-MSCs) and umbilical cord MSCs (UC-MSCs) extracted and cultured in our lab were grown as the MSC cell lines. Induced MSCs (iMSCs) were also utilised. iMSCs were generated from induced pluripotent stem cells (iPSCs) cultured in the lab through two methods: embryoid bodies (iMSC-EB), and direct induction (iMSC-ind). Two iPSC clones were used to generate iMSCs via each of these methods: clone 1 (C1) and clone 27 (C27).

It is most effective to collect CM from cells grown in as large a flask as possible in order to increase the concentration of secreted products in the CM. For example, a 6 well plate has 9.6cm<sup>2</sup> of area for cells, with a media volume of 2ml, whilst a T75 flask has an area of 75cm<sup>2</sup> with 8ml of media, so any CM from a T75 would be twice as concentrated as that collected from a 6 well plate. Although NBL6 and MSC cells were easily grown in a T75, iMSCs (particularly iMSC-ind C27) did not healthily propagate in a T75. Therefore, whilst NBL6 and MSCs were grown in T75s, iMSCs were grown in T25s, and seeded at double the density of NBL6 and MSCs to attempt to counteract some of these changes in CM concentration.

Another step to increase the health of the cells when collecting the CM was to ensure a stepwise reduction in media foetal bovine serum (FBS) concentration. Whilst cells in culture are normally grown in the presence of (20%) FBS to promote their health, CM must be FBS-free if it is to be used therapeutically to reduce its immunogenicity when injected. It was determined that rather than immediately changing the cells from 20%

to 0% FBS, cell viability increased if cells were incubated in 20% FBS for 24 hours, then changed to 10% FBS for 24 hours before moving to the final 0% media.

Then, we investigated how adding CM to bacteria impacts on their growth. The growth of *Staphylococcus* and *Streptococcus* was significantly inhibited in the presence of CM, showing that CM has antimicrobial effects against clinical equine isolates. One exception was that C27 iMSC-CM did not reduce *Streptococcus* growth, which is possibly a reflection of the reduced health of the C27 iMSCs. Interestingly, *Escherichia coli* isolates did not respond to MSC-CM, with no significant changes in growth rate, potentially suggesting a specific effect of this CM on gram-positive cocci.

CM was also combined with sublethal concentrations of antibiotics (to which the bacterial strains were susceptible) to determine whether CM potentiates the effect of antibiotics and therefore could help treat persistent infections. Further optimization of antibiotics may be required in those experiments to produce an effect, as we have observed positive results in other species when CM was combined with antibiotics.

In conclusion, CM can be collected from a range of MSCs, including those generated in the lab from iPSCs. Importantly, CM has antimicrobial effects against equine clinical bacteria isolates from two common species (*Staphylococcus aureus* and *Streptococcus equi zooepidemicus*), which are often more robust against antibiotic uses than laboratory strains of the same species. These are very promising results and suggest that CM from MSCs could be used as an alternative, or in combination with antibiotics, to treat complex MDR infections in horses.

## Implementation of Qualitative Behaviour Assessment as a tool to improve racehorse welfare/Quality of Life (QoL)

Principal Investigator:	Gemma Pearson
Administering Institution:	The University of Edinburgh, UK
Amount Awarded:	GBP 207,312 - Major Research Grant 2024
Project Period:	June 2025 - June 2028
Grant Reference No.:	MRG-241049
Status:	Active
Field of Research:	Equine welfare / Tools to quantify a horse's welfare status / Studies into management practices that impact the welfare of racehorses / Equine behaviour

*Brief Summary: The development and application of Qualitative Behaviour Assessment (QBA) aims to provide a simple and reliable tool to monitor animal welfare states, that can be used by racing stakeholders to evaluate equine emotional expression, addressing the priority to 'identify common factors that improve or degrade the welfare of racehorses'.*

Animal welfare, and what society perceives as the acceptable ethical treatment of animals (sometimes called 'social licence to operate'), is an important part of any activity involving the use of sentient animals. Animal welfare can be defined as the balance between positive and negative emotional states and can span very good welfare to very poor. Good animal welfare, or a good QoL, occurs when animals feel well and have positive experiences more often than they experience negative emotional states. In order to be able to measure the balance of positive to negative welfare states, and to understand what animals do want, and what they do not, we need reliable, robust and validated tools. In our previous research, we demonstrated that a novel behavioural assessment tool, Qualitative Behaviour Assessment (or QBA), provided a valid and practical method to assess the welfare of Thoroughbred racehorses. QBA is a scientifically validated way to use the horses 'body language' as a method to assess their emotional state. Unlike other methods, QBA does not require the researcher to infer what the animal might be feeling, and it is able to measure both positive (such as contentment, relaxation, or positive engagement) and negative states (such as fearful, anxious or uncomfortable). In this project we will extend these results to develop a customised mobile phone-enabled App for collecting QBA data for racehorses. These data will then allow us to track the welfare state of individual animals over time, and to understand the different contexts and situations in which racehorses might feel more positively, and which situations they may find more challenging. The evidence generated can be used to

manage horses in a way that maximizes the balance of positive to negative experiences and promote optimal QoL. We will also investigate whether using QBA causes stakeholders to think more positively about and engage in monitoring horse welfare. Overall, the project will investigate the use of our novel welfare assessment approach, QBA, to identify situations where horses can experience good welfare states. In summary, we will investigate if the unique aspects of QBA can identify simple changes that can make a big difference to a racehorses QoL.

## Looking on the brighter side of life – Characterising the expression of positive emotion in Thoroughbred horses

Principal Investigator: Hayley Randle  
Administering Institution: Charles Sturt University, Australia  
Amount Awarded: AUD 325,693 - Major Research Grant 2022  
Project Period: January 2023 - January 2026  
Grant Reference No.: MRG-2022-100032  
Status: [Active](#)  
Field of Research: Well-being of the racehorse / Tools to quantify a horse's welfare status / Neuroscience / Equine welfare / Equine behaviour

This project will identify and validate behavioural indicators of positive emotions in horses that can be used in industry to improve the welfare and quality of life of Thoroughbred racehorses via an emotional Quality of Life (EQoL) assessment tool. Horse welfare fundamentally depends on what horse's experience and how having to cope with the challenges and opportunities in their environment affects them. Research in other species has shown that behavioural responses to challenges and opportunities are driven by emotions, that is, how the animal feels. Despite the wide availability of industry advice about how to use horse behaviour to assess specific emotional states, there is relatively little scientific evidence to substantiate this advice which means that there is wide variation in how common behavioural responses of horses are interpreted in industry. Very little is known about behavioural indicators of positive emotions in horses and the absence of signs of negative emotions or welfare does not mean the animal is in a positive emotional or welfare state. Therefore, there is a need to identify reliable indicators of positive emotions and emotional state in horses. Being able to reliably identify when a horse is in a positive emotional state will help those responsible for racehorses to improve quality of life by maximising positive welfare across all phases of the racehorse's life.

This project will firstly collaborate with industry to identify the indicators currently used to identify equine emotional states. Then a series of studies adapted from cognitive neuroscience and psychology will identify behavioural indicators associated with positive emotions in Thoroughbred horses to develop the EQoL assessment tool. Horses will experience beneficial outcomes, such as the receipt of food rewards, and the behaviours and physiology associated with those outcomes will be used to develop the EQoL. In particular horses will learn to make predictions about the likelihood of receiving tasty food rewards or associating a specific visual symbol or location with either getting or not getting a reward. The receipt of rewards are associated with behavioural and physiological responses that indicate positive emotions such as happiness in other species. In our project, the behaviour and basic physiological responses of the horses during these tests, as they successfully or unsuccessfully predict their chances of getting a reward will be closely analysed to identify the specific behaviours that occur when they receive rewards compared to when they do not. The behaviours associated with the beneficial outcomes will form the basis of the development of the EQoL indicators that will be tested in industry settings across multiple countries to ensure they can be successfully applied to identify positive emotional states in racehorses. Finally, the findings of these studies, and in particular, the descriptors of the behavioural indicators will be adapted for sharing with the horse industry to provide a powerful and evidence-based EQoL tool for the detection of positive emotions in horses. With this information, industry participants will be able to maximise positive emotions and positive welfare in Thoroughbred racehorses.

## The development and validation of novel behavioural assessment methods for equine welfare

Principal Investigator: Catherine Dwyer  
Administering Institution: The University of Edinburgh, UK  
Amount Awarded: GBP 24,627 - Pump-prime Funding 2021  
Project Period: February 2022 - February 2023  
Grant Reference No.: PPF-2021-101873  
Status: **Completed**  
Field of Research: Animal welfare / Equine behaviour

### Plain Language Summary

Animal welfare is increasingly seen to be important but our ability to recognize good or poor welfare needs robust, scientifically validated measures to ensure that good decisions are made about the quality of animal lives. Most concepts of animal welfare consider that welfare is about what the animal experiences, both positive and negative (e.g. contentment, excitement, hunger, pain etc.). Our stakeholder engagement research has demonstrated that people working in horse racing in various roles, such as trainers, riders, grooms, and veterinarians, have a similar view of what racehorse welfare is to these scientific concepts. The focus of this work has been to identify and test whether we can use behavioral methods to assess horse welfare, in all the situations that may occur for a racehorse, based on identifying those methods that seem to reflect equine emotional states. We used a novel form of behavioral assessment, Qualitative Behaviour Assessment (QBA) which describes the behavior of a horse in terms of how the animal behaves, rather than what it does. This means that the emotional expression, or body language, of the horse provides information about the underlying mood state. Using short video segments collected from Thoroughbred horses on several different racing yards in different contexts, we developed a list of terms and definitions that described the range of emotional states that horses can experience. These were developed by a group of observers and discussed and agreed with racing stakeholders. We tested and validated the ability of QBA to assess equine emotional state by conducting a series of standardized tests to manipulate equine emotions. We found that QBA assessments were able to reliably distinguish between different emotions and these were meaningfully related to other detailed, and time-consuming, quantitative behavioral assessments. QBA assessments of horses experiencing positive emotions were associated with the time horses spent eating and with relaxed ear and head positions, whereas negative mood states were associated with vocal or avoidance responses. Horse activity was associated with increased intensity of the underlying emotion, but did not provide information on whether the emotional state was positive or negative. QBA is easy and quick to apply, had good acceptability with the racing stakeholders, and provides a useful tool to reliably assess equine emotions. This can allow further work to address aspects of horse management that provide more positive emotions, and to avoid or improve areas which elicit poor emotions, thus improving the welfare of Thoroughbred racehorses.

## Potential for racehorses to adapt to different careers on retirement from racing

### Transforming retraining: using multidisciplinary expert consensus to improve success rates in racehorses' second careers

Principal Investigator: Jane Williams  
Administering Institution: Hartpury University, UK  
Amount Awarded: GBP 27,830 - Pump-prime Funding 2022  
Project Period: January 2023 - June 2024  
Grant Reference No.: PPF-2022-100033  
Status: Completed  
Field of Research: Well-being of the racehorse / Retirement and careers beyond racing / Equine welfare / Retirement from racing / Equine behaviour

#### Presentation:

Williams, J. (2024, Jul 10). Transforming Retraining: Supporting racehorses to have successful second careers. Vimeo. <https://vimeo.com/981916277?share=copy>

### Plain Language Summary

Optimizing long-term outcomes for former racehorses to provide them with suitable homes after they leave racing is a priority across the horseracing industry. Following their racing career, retirement often requires racehorses to adapt to a new lifestyle and this transition requires considerable physical and mental adaptation on the horse's part. An optimal early retraining process during this 'transition' can maximise racehorses' potential to have a good quality of life and successful second careers beyond racing.

While many specialist retraining centres and equestrian professionals have significant experience in managing the transition process, most racehorses are not rehomed via specialist centres. Therefore, outcomes for all former racehorses entering their second careers could be improved by identifying the common challenges of retraining and the common features of successful retraining from experienced retrainers and associated veterinary professionals.

Initially we surveyed Racehorse retrainers around the world to identify features key that underpin successful retraining. Most respondents were based in Great Britain or Australia. Four areas were identified as influential 1) time, 2) people involved, 3) horse's history, and 4) approach to retraining. These results informed a Delphi consultation, where experienced retrainers and musculoskeletal therapists, provided in depth feedback to agree specific areas required in retraining to ensure successful outcomes. An individual approach to retraining centered on the horse's needs was clearly articulated as important. Seven areas were rated as essential to a successful transition: feeding regime, the rider, the handler, the type of exercises undertaken, assessing/changing the horse's shape, assessing/changing the horse's posture, and provision of turnout. The thoroughbred's versatility and ability to excel in a range of careers was evident across both stages of the project, with retrainers commonly preparing horses for eventing, dressage, and leisure homes.

Matching horses' capabilities with owner expectations was considered essential to successful transition by retrainers. Increased education of owners to support this alongside increased industry recognition and financial support for retrainers were highlighted as areas that could be developed to support positive transition outcomes for horses. Applying a multidisciplinary approach, combining the expertise of professionals was also identified as a method that could support transition and further promote successful second careers.

The results provide important insights into how key stakeholders in former race-horse retraining manage the transition of horses from leaving racing to securing a viable second career homes. Across the survey and the Delphi study, the industry's focus on placing the horse and their needs in the centre of a flexible transition process was fundamental to success. A potential framework that could support transition emerged from our results; this dynamic model places the horse at the centre and integrates the expertise of professionals involved in transition across four fundamental steps: 1) review of the horse's history; 2) initial assessment; 3) regular progress assessment during re-training; and 4) owner guidance to facilitate suitable matches, to optimise successful future careers that have the potential to provide a good life for former racehorses. Further

development of this model could improve horse welfare and support transition for less experienced owners and handlers.

### **Key Findings and Outcomes of the Project**

Following their racing career, retirement requires racehorses to adapt to a new lifestyle which requires considerable physical and mental adaptation from the horse. Identifying an optimal transition is paramount to maximize a good life beyond racing.

A survey of global stakeholders (n=100) experienced in retraining racehorses completed an online survey to identify common themes and challenges associated with the transition from racehorse to successful second careers. These results informed a subsequent Delphi consultation with retrainers, and musculoskeletal therapists (n=27) experienced in supporting racehorses to successful second careers. Participants were recruited through racing social media, industry contacts and snowball sampling. Survey data were analyzed using frequency analysis and conventional inductive content analysis. In addition, for the Delphi, content validity ratios determined consensus for behavioral, physical, exercise and management factors that should feature in transition.

Survey respondents identified 4 themes key to successful retraining: 1) time, 2) people, 3) horse's history, and 4) approach. Across the survey and the Delphi study, the industry's focus on placing the horse and their needs in the centre of the transition process was evident, with the flexible nature of the process of transition fundamental to success. The findings indicate that transition timeframes are variable and can take up to 2 years. The Delphi identified seven areas essential to transition: feeding regime, the rider, the handler, type of exercises undertaken, assessing/changing the horse's shape, assessing/changing the horse's posture, and provision of turnout. Our results suggest retrainers are predominately producing horses for leisure, dressage and eventing second careers with groundwork, the importance of the handler and adapting horse's management fundamental to successful transition.

Across both stages of the study, respondents applied a subjective rather than objective approach to the assessment of horses' progress throughout transition. The soundness, health and gait of a horse combined with how they are mentally coping with training and assessment of their physical development were advocated to evaluate a horse's progress through transition. Few people used available tools and technology to support this suggesting an opportunity exists to embed a more evidence-informed approach to progress assessment within transition utilizing the input of different professionals to facilitate this.

The importance of people, those actively involved in retraining and the future owners and keepers of horses after transition, was a consistent theme across all stages of the study. Successful transition appears to be defined as securing the right match between the horse and its new owner or keeper, which aligns both the horse's physical, mental and training capabilities and the owner's expectations providing the horse with a good life moving forward. These results should inform the development of objective, multidisciplinary team, evidence-informed pathways that can support retrainers and future owners to facilitate suitable second careers for individual racehorses after transition.

Across this work, a potential framework has emerged that could support transition. This dynamic model places the horse at the centre and integrates the expertise of professionals involved in transition across four fundamental steps: 1) review of the horse's history; 2) initial assessment; 3) regular progress assessment during retraining; and 4) owner guidance to facilitate suitable matches, to optimise successful future careers that have the potential to provide a good life for former racehorses. Future work should seek to develop this framework to generate tools that can be shared with industry stakeholders and potential owners and keepers to support successful second careers and improved quality of life for former racehorses.

## The biological integrity of the horse

### MULTITRACE MS - MULTIplexed TRANsgene deteCtion in the Equine by Mass Spectrometry

Principal Investigator:	Mario Thevis
Administering Institution:	German Sport University Cologne, Germany
Amount Awarded:	EUR 327,735 - Major Research Grant 2024
Project Period:	February 2025 - February 2028
Grant Reference No.:	MRG-241014
Status:	Active
Field of Research:	Protection against artificial manipulation of performance / Techniques to detect manipulation of an individual horse's genetic blueprint / Gene doping / Molecular biology

*Brief Summary: The proposed project aligns with the priority research topic of the Foundation: "Detection of alteration of the equine genome or biomarkers that indicate artificial manipulation of performance".*

Horse racing is not only a sports discipline fascinating fans of equestrian sports worldwide but also a billion-dollar business (in 2019 the International Federation of Horseracing Authorities (IFHA) estimated an overall betting turnover of about 115 billion € in the participating countries). Altering of the constitution and expression of genes by gene therapy could offer the unprecedented opportunity to instantly optimize inherent traits in horses and its increasing application in equine professional sports for performance enhancement (known as "gene doping") can be anticipated. Illicit genetic manipulations in equine professional sports could coerce the integrity of horse racing, provoke unfair wagering and, if not sufficiently clinically tested, could pose profound and unpredictable risks on the health and welfare of horses. This project intends to develop a new gene doping detection method which will provide a straight-forward tool to detect a variety of artificial genes of which either one could be transferred into a horse in a gene doping attempt, e.g. to promote muscle growth or to increase muscle oxygen-supply in order to enhance the horse's strength and endurance. The proposed detection method is intended to make a meaningful contribution to gene doping analysis in horses, the fairness of equine professional sports, and the welfare of sports horses.

## Others

### **Equine influenza virus epigenetically imprints airway basal cells and alters chronically the airway epithelium repair potential.**

Principal Investigator:	Caroline Chauche
Administering Institution:	The University of Edinburgh, UK
Amount Awarded:	GBP 27,582 - Pump-prime Funding 2021
Project Period:	February 2022 - January 2023
Grant Reference No.:	PPF-2021-101197
Status:	Completed
Field of Research:	Virology / Respiratory medicine / Respiratory health and disease / Infectious disease / Molecular biology / Genetics

### **Plain Language Summary**

Equine influenza virus (EIV) is an important respiratory pathogen that causes a severe disease in horses and poses a constant and serious threat to the welfare of racehorses. EIV infects cells in the horse lung and causes their death, creating microscopic holes in the process that needs to be promptly repaired. If this repair is deficient, the horse will take longer to recover, and its lungs might get permanently damaged. Identifying a dysfunctional lung repair response in a young racehorse exposed to flu is essential to better protect it and avoid long-term lung damage. Recent advances in EIV vaccine research have led to the generation of temperature-sensitive Live Attenuated Vaccines (LAIV), which seem to efficiently protect horses against EIV, but little is known about their effect on respiratory cell biology.

EIV infects different cells in the lung, and notably some called respiratory basal cells, whose role is to repair the lung after injury. They are instructed to do so by repair molecules that make them multiply and transform into other lung cell types that EIV has just destroyed. Interestingly, the repair molecules are also involved in initiating the production of defense molecules responsible for alerting the body that it is infected by a virus. Scientific information obtained in mouse and human indicates that when the flu virus infects respiratory basal cells in the mouse it blocks the repair molecules, which prevents the repair of the damaged lung. To date, it is unknown if EIV or LAIV block repair responses during infection.

To evaluate if EIV and LAIV modify the respiratory repair responses following an infection, we have infected horse respiratory cells in the laboratory with EIV and LAIV and compared the biology of all cells present in culture. We have identified key differences between cells that have not been exposed to a virus compared to those that have been exposed to EIV or LAIV, as well as key differences in the type of cells that constitute the respiratory epithelium upon exposure with EIV or LAIV.

Equine flu is a huge welfare and economic burden for the Thoroughbred and the equine industry, and our goal was to provide insight in the effect of EIV on respiratory cells biology and help evaluate how LAIV may help alleviate this burden. Understanding how a dysfunctional repair response leads to long-term alteration of the horse lung is an essential step towards the design of adequate vaccines that protect the lungs of young racehorses exposed to flu. Our project has paved the way for the development of novel therapeutics to promote healthy lung repair and guarantee prompt and safe return to training and racing activities after viral exposure. Developing such novel knowledge will likely have a significant positive impact for both the welfare and long-term athletic career of Thoroughbreds.

## Host immune response against EHV-1: a novel approach.

Principal Investigator:	Lutz Goehring
Administering Institution:	University of Kentucky, US
Amount Awarded:	USD 344,647 - Major Research Grant 2024
Project Period:	March 2025 - November 2027
Grant Reference No.:	MRG-241030
Status:	<a href="#">Active</a>
Field of Research:	Infectious disease / Control and prevention of current and emerging infectious diseases / Identification of risk factors associated with infectious disease / Immunology / Neurological disease / Virology / Immunopathology

*Brief Summary: Our understanding of EHV-1 etiopathogenesis and horse immune response is rudimentary, while there is an inherent risk for adult Thoroughbred horses to develop neurological complication due to spinal cord disease upon infection with the virus. There is a dire need for a better understanding of host-pathogen interaction.*

Equid alphaherpesvirus-1 (EHV-1) infection causes mild to moderate respiratory disease in horses but is feared because of complications like spinal cord disease causing ataxia and paralysis, or abortion in the pregnant mare. Spinal cord disease can result in loss or can cause temporary or permanent damage. It is a highly contagious disease. As secretions of the respiratory tract of a shedder contain high viral loads, an identified outbreak will lead to quarantine of the facility; closure of racecourses or venues, and cancellation of events. Our understanding of pathogenesis and host immunity is rudimentary; particularly, on the pathogenesis of myeloencephalopathy (EHM) or spinal cord disease. In order to develop EHM in a horse, there has to be a blood-borne transportation phase, viremia. Most information so far has been gathered from in vitro studies; outbreak descriptions; few epidemiological studies, and from several experimental infection studies with the main goal to compare a treated (or vaccinated) group of horses with a control group. A high dose of infectious virus is instilled into the pharynx of a horse, and the clinical course, nasal shedding, viremia and changes in immune response are studied day-by-day. The question remains, if this is an unrealistic, possibly overwhelming dose to model this disease! Field outbreaks of EHV-1 with EHM typically have the most (severely) affected cases at the beginning/first half of an outbreak, with incidental cases only during the second half. We believe that these findings are dose, dose-over-time, transmission-dependent, and developing/pre-existing immunity. We further believe that there is low-dose immune system priming during the initial phase of an outbreak in horses that are not in direct contact with or at a greater distance with a shedder. We want to test the hypothesis that dose differences in quantity or quality, or differences in deposition an infectious dose within the horse's respiratory tract will alter clinical outcome and the immune response. Aims are to compare alternative models of infection with the current standard: i) high dose (standard) vs low dose; ii) upper respiratory (standard) vs lower respiratory tract infection, and iii) airborne transmission (distance). Aim 1 and 2 will be conducted in a state-of-the-art individual boxes/stalls BSL-2 facility, while aim 3 will be conducted in a temporary stable construction with a single shared airspace. Measurable outcome will be differences between groups in viral nasal shedding, fevers, duration and magnitude of viremia, and immune parameters. Unique in aim 3 will be if a distance of 4m between 2 horses (one being a shedder) is enough to prevent infection or to test if there is (sub)clinical infection with immune system priming of the in contact. We will also test whether priming will prevent infection. Furthermore, we will test if condensation is a potential source for transmission and environmental contamination. Impact: this study will answer important questions on how we will/should perform infection experiments in the future, and this study will impact current opinion on measures that are implemented during EHV-1 outbreaks.