

### **3 year full time PhD Scholarship at the University of Edinburgh**

#### **Title: Do mycotoxigenic fungi cause equine grass sickness?**

**Supervisors:** Prof Bruce McGorum, Prof R. Scott Pirie and Dr Gerry McLachlan

**Funding:** The Horse Trust ([www.horsetrust.org.uk](http://www.horsetrust.org.uk))

**Background:** Equine Grass Sickness (EGS; also known as equine dysautonomia) is a predominantly fatal multi-system neuropathy of grazing horses that kills approximately 1-2% of UK horses annually. An apparently identical disease occurs in cats, dogs, hares, rabbits, llamas and possibly sheep. Since it was first reported in 1906, it has been the subject of numerous research projects, but the cause of this enigmatic disease remains unknown. There is no effective treatment or prevention. This project will test the hypothesis that EGS is caused by a neurotoxin produced by a pasture-derived fungus, which is ingested directly from the pasture along with the parent fungus, or released from ingested fungi within the gastrointestinal (GI) tract. Many risk factors for EGS are consistent with involvement of a fungal neurotoxin, including seasonal occurrence, geographical clustering and preceding climatic conditions. Furthermore, the applicants' and previous pilot studies identified an association between EGS and increased numbers of particular fungi on grass. We aim to identify the cause of EGS by demonstrating (a) an increased prevalence of the causative fungus both in the GI tract and in feed from EGS cases compared with controls, and (b) an increased prevalence of fungi expressing genes for biosynthesis of candidate mycotoxins in the aforementioned samples. Identical analyses of GI and feed samples from cats with the feline form of EGS (feline dysautonomia, FD) will provide powerful complementary data. The project will utilise state of the art molecular analysis of the mycobiota (fungal populations) within GI contents from EGS and control horses, FD cats and controls, in feed from EGS/FD outbreaks and control feeds using a targeted amplicon sequencing approach. This approach will combine PCR amplification of fungal Internal Transcribed Spacer 1 (ITS1) sequences from GI samples with next-generation sequencing (Illumina MiSeq platform) and a bioinformatic pipeline to map the identified sequences using a fungal DNA database such as the Targeted Host-associated Fungi (THF) database (Tang et al 2015). We will also perform targeted screening of GI and feed samples for expression of mycotoxin biosynthetic genes associated with production of specific mycotoxins, selecting likely targets based on their previously demonstrated toxicity profile and whether their potential involvement is consistent with published risk factors for EGS. The student will gain expertise in the relatively new and expanding fields of microbial community analysis and bioinformatics, both of which have translational applications in the investigation of a wide range of equine health and welfare issues. The student will also be involved in sample collection and visits to affected pastures.

**Aim:** The study will extend the applicants' unpublished pilot data to further test the hypothesis that EGS is caused by neurotoxins produced by environmental mycotoxigenic fungi.

#### **Objectives:**

1. Characterise mycobiota (fungal population) using a targeted amplicon sequencing approach within;

- a) Gastrointestinal (GI) contents collected *post mortem* from multiple sites throughout GI tracts of EGS and grazing control horses
  - b) GI contents collected *post mortem* from multiple sites throughout GI tracts of cats with feline dysautonomia (FD; feline form of EGS) and control cats
  - c) Food samples associated with dysautonomia cases, including herbage collected from EGS and control fields immediately after an occurrence of EGS, silage that was being fed to a horse without pasture access when it developed EGS, control silage, cat food collected following FD outbreaks and control cat food.
2. Screen by PCR for specific candidate causative fungi identified in (1) (ie those exclusively present and/or more prevalent in EGS-associated samples) to further determine the prevalence of these fungi in larger numbers of GI and food samples.
  3. Determine the prevalence of fungi expressing genes for biosynthesis of selected candidate mycotoxins, within GI and feed samples, by RT-PCR using mycotoxin biosynthetic gene primers. Candidate mycotoxins will be selected based on data from (1) and (2), applicants' pilot data, published toxicity profiles and consistency with known EGS epidemiological factors.
  4. Provision of high quality training in a world leading scientific institute and development of expertise in microbial community analysis and bioinformatics, a rapidly growing field which has increasing utility in investigations of equine health (multiple body systems) and welfare.

Identification of the cause of EGS would be a significant breakthrough, since this could inform new methods to prevent, treat and diagnose this devastating disease. Consequently, this project could have significant benefits for equine health and welfare

The successful applicant will join the McGorum, Pirie and McLachlan groups (links below) at The Roslin institute, and at the Dick Vet Equine Hospital, University of Edinburgh.

[www.roslin.ed.ac.uk/bruce-mcgorum/](http://www.roslin.ed.ac.uk/bruce-mcgorum/)

[www.roslin.ed.ac.uk/scott-pirie/](http://www.roslin.ed.ac.uk/scott-pirie/)

[www.roslin.ed.ac.uk/gerry-mclachlan/](http://www.roslin.ed.ac.uk/gerry-mclachlan/)

**Eligibility:** The student must hold a relevant veterinary degree (which permits work as a veterinary surgeon in the UK), or an appropriate science degree from a UK or EU university. In exceptional circumstances candidates from non-EU countries will be considered. The candidate should wish to pursue a research career in the equine field to improve welfare of equids.

**Start date:** between September 2016 and January 2017.

**Salary:** Qualified veterinarians will receive a tax-free stipend of £21,000 annually for 3 years. Student fees will be paid by the sponsor.

**Contact:** Please contact Bruce McGorum ([bruce.mcgorum@ed.ac.uk](mailto:bruce.mcgorum@ed.ac.uk); 44-131-650-6253/6230) or Scott Pirie ([scott.pirie@ed.ac.uk](mailto:scott.pirie@ed.ac.uk); 44-131-650-6253/7230)