

PROJECT/STUDY TITLE:	Randomised controlled trial of <i>Clostridium botulinum</i> type C vaccination for the prevention of Equine Grass Sickness.
PRINCIPAL APPLICANT(S)	Dr Jo Ireland Co-applicants: Professor Bruce McGorum; Dr Richard Newton; Professor Christopher Proudman; Professor Debra Archer
GRANT AWARDED (DATE):	10th June 2013 (Project commenced January 2014; AWF-funded phase NHF_2013_06_JI commenced March 2014)

Lay summary of project outcomes, achievements and potential impact: Max 300 words

Equine grass sickness (EGS) is a predominantly fatal disease affecting grazing equids. Research suggests that EGS is a toxico-infection involving *Clostridium botulinum* (*C. botulinum*), with several studies demonstrating a protective effect of natural immunity to *C. botulinum* type C. Other clostridial diseases are successfully prevented by vaccination, implying that it should be possible to prevent EGS by vaccination.

This project will determine the efficacy of *C. botulinum* type C vaccination in preventing EGS by performing a nationwide randomised controlled field trial, comparing EGS incidence between groups of vaccinated and placebo-treated horses/ponies.

Recruitment for the EGS vaccine trial commenced in March 2014, enrolling privately owned horses/ponies from EGS-affected premises. Only horses/ponies over six months old, with a valid passport, and kept on premises with a history of at least one EGS case within the preceding three years were eligible for enrolment.

A total of 120 premises are participating in the trial (52% in England and 48% in Scotland), with 84 veterinary practices recruited as site investigators. A total of 1,001 eligible horses and ponies were enrolled by 221 owners. On enrolment, these horses/ponies were randomly selected to enter either the vaccine or placebo treatment group. Of the enrolled animals, 936 horses/ponies have completed the entire primary treatment course (comprising three vaccine or placebo injections administered at 21 day intervals) to date. For horses/ponies enrolled during 2014, annual booster administration commenced in April 2015, and these animals will receive a second annual booster in 2016. Three confirmed EGS cases have occurred within the study population to date. Preliminary interim analyses indicated that an extension to the EGS vaccine trial study period to include a third year is required to meet sample size requirements.

Masking of treatment group allocations will be maintained until final data analyses are completed in early 2017, where the incidence of EGS will be compared between the vaccine and placebo treatment groups.

Demonstration of reduced disease incidence in vaccinated animals would provide a major breakthrough in EGS prevention, with an effective vaccine representing the first preventive healthcare measure to reduce the impact of this disease.

Detailed progress against original objectives: List outcomes against original objectives. Discuss what has been achieved, including any statistical analysis completed as part of the project.

Objective 1: To quantify the protective effect of vaccination by comparing EGS incidence rates in horses receiving *C. botulinum* type C toxoid vaccination and placebo-treated control horses.

Project Outcomes: The primary outcome of this study will be determining the efficacy of *Clostridium botulinum* (*C. botulinum*) type C toxoid vaccination in the prevention of naturally

occurring EGS. Recruitment for the nationwide triple-blinded randomised, placebo-controlled field trial (RCT) commenced in March 2014. In May, the Veterinary Medicines Directorate (VMD) authorised amendments to the Animal Test Certificate protocol, which included changing from the originally specified closed cohort to an open cohort with a rolling recruitment phase.

A total of 84 veterinary practices were recruited as site investigators and 120 EGS-affected premises meeting inclusion criteria were recruited. Of these, 52.5% of premises are in England the remaining 47.5% are in Scotland, with a median EGS incidence at baseline of 2.3 cases per 100 horse-years at risk. By a total of 221 participating owners, 1,025 horses/ponies were enrolled, of which 1,001 meeting all inclusion criteria were randomised at a 1:1 ratio to either the vaccine or placebo treatment group. Of the enrolled animals, 40 were withdrawn/excluded prior to the first treatment administration and 936 horses/ponies completed the entire primary treatment course (comprising three injections of vaccine or placebo at 21 day intervals).

Owner-reported safety data is obtained, for a seven day period following each RCT injection, via owner-completed post-treatment observation recording forms. To date, no serious adverse reactions have been reported. Thirteen veterinary-attended adverse events have been reported to date, of which seven were injection site reactions considered likely to be related to trial injection administration, including both trial product-related or procedure-related e.g. mechanical or microbial injury.

For animals enrolled during 2014, annual booster injection administration commenced in April 2015, and to date, 448 horses/ponies have received the annual booster. Health and management monitoring will be continued via regular telephone follow-up questionnaires, and this subset of animals will receive a second annual booster in 2016. The overall retention rate following randomisation and the first treatment administration is 87.9%. Three histologically confirmed EGS cases have occurred within the study population to date, representing an EGS incidence of 0.28 cases per 100 horse-years at risk. This incidence rate is lower than that of the sampled population at baseline (median EGS incidence at baseline of 2.3 cases per 100 horse-years at risk), possibly reflecting the temporal variation in EGS incidence between years (Wylie *et al* 2011).

The first scheduled interim analysis was completed in April 2015, with an interim report issued to the VMD in May 2015. The median EGS incidence rate on trial sites at baseline was consistent with published incidence rates, and with *a priori* sample size calculations, indicating that the recruitment methodology successfully identified high risk premises. No systemic adverse reactions were reported, and the incidence of injection site adverse reactions was low. No interactions between the test product vaccine or placebo and any concomitant treatments have been reported to date. There was no significant difference in hazard of veterinary-attended product-related adverse reactions between treatment groups ($p=0.46$). Based on the single EGS case included in the first interim analysis, there was no significant difference in the risk of EGS between treatment groups ($p=0.62$). The median animal age at enrolment was 8.09 years (IQ 5.00 – 13.94 years; range 0.50 – 29.00 years). The study population comprised 54.3% male animals ($n=358$; including 51.4% geldings, $n=339$ and 2.9% colts/stallions, $n=19$) and 45.7% females ($n=301$; including 37.8% mares, $n=249$; and 7.9% fillies, $n=52$). The most numerous breeds were Irish Draught/Irish Draught cross breeds/Irish Sports Horse (19.0%, $n=125$), Native/Native cross breeds (18.5%, $n=122$), Welsh/Welsh cross breeds (14.1%, $n=93$), and Cob/Cob cross breeds (13.1%, $n=86$). There were no significant differences between treatment groups for age ($p=0.28$), gender ($p=0.66$) or breed ($p=0.87$) distributions, indicating that the randomisation method used was successful in controlling for horse-level risk factors for EGS. All enrolled animals were found to be in good general health at the first veterinary visit.

An additional interim analysis will be undertaken in April 2016, and will include descriptive statistics on the study population together with assessment of the incidence of adverse reactions and EGS overall and between treatment groups. Blinding of all RCT investigators will be maintained during both serological assays and interim analyses.

Objective 2: Demonstrate immunological responses to *C. botulinum* type C toxoid vaccination using pre- and post-vaccination serological assays.

Project Outcomes: The Animal Health Trust's Immunology Group has established an enzyme-linked immunosorbent assay (ELISA) for quantifying pre- and post-vaccination antibody titres to *C. botulinum* type C antigens. Laboratory staff performing ELISAs are blinded to animals' treatment group allocations throughout the entire study period.

To date, paired serum samples have been collected from 898 enrolled horses/ponies, obtained prior to administration of the first vaccine/placebo injection and at a median interval of 14 days following the final (3rd) injection of the primary treatment course, and serological analyses of these archived samples are on-going. To maintain blinding of laboratory staff, serology results from a subset of samples have been assessed by the unblinded person responsible for maintaining the treatment group allocation data. All serological data assessed were consistent with treatment group allocation and there was clear evidence of seroconversion in a number of animals within the subset of samples assessed. Administrations of booster injections commenced in May 2015, and to date, paired serum samples have been collected from 398 horses/ponies, obtained prior to administration of the first annual booster vaccine/placebo injection and at a median interval of 14 days following the booster injection. Analysis of paired pre- and post-booster serum samples is due to commence imminently.

Reference:

Wylie, C.E., Proudman, C.J., McGorum, B.C. and Newton, J.R. (2011) A nationwide surveillance scheme for equine grass sickness in Great Britain: Results for the period 2000–2009. *Equine Veterinary Journal* **43**, 571-579.

Were there any challenges or barriers/modifications to the project? Explain the nature of and reasons for any changes in project focus, scope, delivery, schedule or evaluation.

Project start date

The recruitment phase of the RCT (including the NHF-funded phase) was intended to commence in January 2014, and a database of potentially eligible owners was compiled during 2013 from respondents to the preceding feasibility questionnaire survey, EGS surveillance scheme and the EGS Fund database of EGS-affected premises where owners expressed an interest in participating. The Animal Test Certificate authorising the RCT was approved by the VMD on 17th February 2014. In order to ensure sufficient funding was secured and the required research agreements were completed, the recruitment phase commenced on 18th March 2014, with this delay resulting in a minor change to the schedule of the project.

Recruitment of eligible horses

A total of 558 horse owners were sent, or contacted the study team to request, information regarding the EGS vaccine trial, and >2,500 horse enrolment forms were mailed to owners and veterinary practices. Despite this, recruitment of eligible horses/ponies for the trial took considerably longer than anticipated. In May 2014, the VMD approved amendments to the RCT protocol that included changing from the original closed cohort design to an open cohort with a rolling recruitment phase. The recruitment phase was extended, with recruitment of new trial sites ceased in September 2015. It is widely recognised that patient recruitment is one of the greatest barriers in the conduct of RCTs in human medicine; therefore a protracted recruitment phase for this project was not unexpected. However, given the short recruitment phase for the preceding pilot vaccine trial, and the availability of an existing database of owners interested in participating in the trial, the extent of the delay in reaching the target size of study population was far greater than anticipated.

Achieving target sample size

In order to accrue the required number of horse-years at risk estimated by *a priori* sample calculations (1,800 horse-years at risk), the study period has been extended to include a third year. In December 2015, the VMD approved an extension of the Animal Test Certificate, authorising continuation of the RCT during 2016. Animals enrolled during 2014 will receive a

further annual booster in 2016. To date, the incidence of EGS within the study population is 0.28 cases per 100 horse-years at risk, with two further clinically suspected cases confirmed as EGS-negative on post-mortem histopathology, and five further clinically diagnosed cases on trial sites affecting horses not enrolled in the vaccine trial. This incidence rate is lower than the estimated incidence for the placebo-treated group used in *a priori* sample size calculations. Data from the Royal (Dick) School of Vet Studies, University of Edinburgh (personal communication Prof Bruce McGorum) and the nationwide EGS surveillance scheme (Wylie *et al* 2011) indicate that EGS frequency varies annually, demonstrating a sinusoidal pattern of incidence. Through extending the RCT during 2016, enrolled horses/ponies will be followed throughout a third 'high risk' season and it is possible that EGS incidence in the study population will increase. Additionally, it could be expected that the administration of a second annual booster to a subset of enrolled animals would result in a greater vaccine effect compared to the assumptions used in *a priori* sample size calculations. An additional interim analysis will be undertaken in April 2016, and will include descriptive statistics on the study population together with assessment of the incidence of adverse reactions and EGS overall and between treatment groups.

Provide details of knowledge transfer activities to date and any future plans/actions.

1) Publication of preceding preliminary studies

As detailed in the full application for NHF_2013_06_JI, prior to the RCT commencing, preliminary feasibility, safety and pilot field trials had been undertaken. The results of these preceding studies have since been published:

Ireland JL, Rash N, Rice J, Hennessy K, McGorum BC, Proudman CJ, Poxton IR and Paillot R (2016) Randomised controlled safety study of a *Clostridium botulinum* type C vaccine for the prevention of Equine Grass Sickness: evidence of vaccine immunogenicity and safety in the horse. *Journal of Equine Veterinary Science* **39**, ppS38.

Ireland JL, McGorum BC, Proudman CJ, Rash N, Paillot R, Rice J, and Newton JR (2016) Vaccination against Equine Grass Sickness: piloting a clinical field trial of *Clostridium botulinum* type C toxoid in the United Kingdom. *Journal of Equine Veterinary Science* **39**, ppS39.

"Feasibility study to inform the design of a randomised, placebo-controlled field trial of a *Clostridium botulinum* type C vaccine against Equine Grass Sickness". Ireland JL, McGorum BC, Proudman CJ and Newton JR (2015) Poster presentation at the 14th International Symposium on Veterinary Epidemiology and Economics, November 2015. Available at:
<http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=d5d67a8d-5068-4f33-a945-81117f6fd7f5&cKey=3931ee34-0069-499b-9b95-28afee6c1de2&mKey=%7b6753AA4B-4EFD-470C-B925-C66F22F0712C%7d>

"Site selection survey to assess the feasibility of a randomised, placebo-controlled field trial of a *Clostridium botulinum* type C vaccine against Equine Grass Sickness". Ireland JL, McGorum BC, Proudman CJ and Newton JR (2015) Poster presentation at the 14th International Symposium on Veterinary Epidemiology and Economics, November 2015. Available at:
<http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=d5d67a8d-5068-4f33-a945-81117f6fd7f5&cKey=aff665d0-0133-4817-8305-2d064e1bf573&mKey=%7b6753AA4B-4EFD-470C-B925-C66F22F0712C%7d>

Ireland JL, Newton JR, Proudman CJ, Thomson K and McGorum BC (2013) Vaccination against Equine Grass Sickness: Piloting a clinical field trial of a *Clostridium botulinum* type-C toxoid in Scotland in 2012–13. *Equine Veterinary Journal* **45**, Supplement 44, pp14.

"Vaccination against equine grass sickness: piloting a randomised placebo-controlled field trial of a *Clostridium botulinum* type C toxoid". Ireland JL, Newton JR, Proudman CJ, Thomson K and McGorum BC (2013) Poster presentation at Society for Veterinary Epidemiology and Preventive Medicine Annual Conference, March 2013.

Additionally, a manuscript entitled “Designing a field trial of an equine grass sickness vaccine: a questionnaire-based feasibility study” has been submitted for publication in the Veterinary Journal, and is currently pending a decision following peer review.

2) Training for participating veterinary surgeons

Online webinar, hosted by Boehringer Academy: <https://www.boehringer-academy.co.uk>

Equine Grass Sickness: The association with *Clostridium botulinum* and potential for vaccination

Veterinary seminars regarding EGS RCT presented at Rosssdales Veterinary Surgeons and Newmarket Equine Hospital, March 2014.

Powerpoint presentation regarding EGS and the EGS RCT provided to several participating veterinary practice for use in client education talks.

3) Articles in veterinary media

Letter regarding EGS RCT published in Veterinary Times, April 2014.

Article regarding EGS RCT published in Veterinary Times, May 2014.

4) Owner talks

- Equine grass sickness presentation at owner education evening, October 2015
- Equine grass sickness presentation at BHS Hertfordshire event, September 2014
- Equine grass sickness presentation at Station House Vets equine client evening, May 2014
- Equine grass sickness presentation at “HorseWatch Suffolk” event, March 2014
- Participating veterinary practice filmed segment for BBC Scotland programme “Landward”, April 2014

5) Articles in equestrian media

- “Funds raised to tackle killer disease” *EQ Life Magazine* December 2015
- Equine grass sickness article in *EQ Life Magazine* April 2015
- “A Step Closer to Equine Grass Sickness Prevention”: Editorial feature in *Scottish Horse* November 2014
- “A step closer to equine grass sickness prevention” *Equine Health* September 2014: 19, pp 46-49.
- “Breakthrough in Equine Grass Sickness Prevention on the Horizon” *Pegasus Magazine* June 2014
- Equine grass sickness vaccine trial article in *BHS Magazine* June 2014
- Equine Grass Sickness in: “New research projects from the Animal Health Trust” *Equine Health* January 2014:15, pp 14-15.
- “Nationwide Equine Grass Sickness Vaccine Trial” in *Equine News*:
<http://d1924370.u22.pipeten.co.uk/wp-content/uploads/2013/10/Equine-News-Spring-Summer-2014.pdf>
- “Equine grass sickness on the rise as vaccine trial gets underway”: *Horse & Hound* March 2014
- “Equine Grass Sickness: how close are we to protecting our horses from it?": *Horse & Hound* August 2014
- “Breakthrough in Equine Grass Sickness Prevention on the Horizon” – education feature in *Arabian Magazine* June 2014
- “Grass Sickness still a major threat!": *Equi-ads* March 2014

- Equine grass sickness vaccine trial feature in Diagnosteq spring/summer 2014 newsletter

6) Future plans

- Manuscript entitled “Utilisation of a nationwide disease surveillance scheme to inform the design of a field vaccine trial for Equine Grass Sickness in Britain” in preparation for submission to Preventive Veterinary Medicine
- Final results of EGS RCT, including AWF-funded phase NHF_2013_06_JI, anticipated to be submitted for publication in a peer reviewed journal during 2017

Provide details of any original peer-reviewed research papers, book chapters and books/monographs that have resulted directly from your work supported by this grant.

No original peer-reviewed research papers, book chapters or books/monographs published to date.

Have any other funding bodies been involved in supporting the development of the work supported by this grant?

- Petplan Charitable Trust: pump primer grant awarded for development of *Clostridium botulinum* serological assays 2012
- Stafford Trust: donation in support of J Ireland’s post 2012 – 2013
- HBLB and The Racing Foundation: research grant awarded for EGS RCT 2014 – 2016
- Neogen Corporation: research grant awarded for EGS RCT 2014 – 2015
- Moredun Foundation EGS Fund: research grant awarded for EGS RCT 2014 – 2015
- Hong Kong Jockey Club: research grant awarded for EGS RCT 2014 – 2015
- EB Moller Charitable Trust: research grant awarded for EGS RCT 2014 – 2015
- Miss A M Pilkington Charitable Trust: donation for EGS RCT 2012 – 2014
- The IVO Trust: research grant awarded for EGS RCT 2015 – 2017
- Petplan Charitable Trust: research grant awarded for EGS RCT year 3 serological assays 2016
- Moredun Foundation EGS Fund: research grant awarded for EGS RCT 2016, including funds raised by BHS EGS fundraising campaign
- Additional donations to Animal Health Trust EGS research, including EGS RCT 2013 – 2015 received from:
 South Essex Insurance Brokers
 Welsh Cob & Pony Society
 Mrs DM France-Hayhurst Charitable Trust
 J and JR Wilson Trust

Has any intellectual property activity has resulted directly from the research funded through this grant to date?

No intellectual property activity to date directly from the research funded through this grant. Please see above for details regarding planned publication of results.

Briefly tell us about the staff who received a salary or stipend from this grant (including yourself) - Name, job title, full or part time

Not applicable. This project did not require funding towards a salary or stipend for a specific appointment. Participating veterinary surgeons recruited as Site Investigators are paid on a fixed fee basis for clinical work undertaken by their practice throughout the RCT study period.

How has the grant contributed to the professional development of the staff named above (including yourself)? Max 250 words

<p>Although salary is not funded through this grant, the principal applicant is currently provisionally registered for the RCVS Diploma of Fellowship by thesis, with the aim of full registration during 2016, and thesis submission during 2017 (Fellowship title: Randomised controlled trial of <i>Clostridium botulinum</i> type C vaccination for the prevention of Equine Grass Sickness).</p>
<p>Have you, or any of the staff included above, received any prizes, awards or commendations as a direct result of the research supported by this grant to date? If yes please give details, including the name of the recipient.</p>
<p>No</p>
<p>If any clinical trials have been supported by the funding of this grant, please enter the title of the trial and briefly describe any key developments or outcomes (Max 300 words)</p>
<p>Clinical trial title: Efficacy of vaccination against <i>Clostridium botulinum</i> type C in the prevention of naturally occurring Equine Grass Sickness, 24 – 30 month duration randomised placebo controlled field trial (RCT)</p> <p>The primary outcome of this study will be determining the efficacy of <i>Clostridium botulinum</i> (<i>C. botulinum</i>) type C toxoid vaccination in the prevention of naturally occurring EGS. Recruitment commenced in March 2014, with 84 veterinary practices recruited as site investigators and 120 EGS-affected premises meeting inclusion criteria recruited. Of these, 52.5% of premises are in England the remaining 47.5% are in Scotland, with a median EGS incidence at baseline of 2.3 cases per 100 horse-years at risk, consistent with published incidence rates, and with <i>a priori</i> sample size calculations, indicating that the recruitment methodology successfully identified high risk premises.</p> <p>A total of 1,025 horses/ponies were enrolled, of which 1,001 meeting all inclusion criteria were randomised at a 1:1 ratio to either the vaccine or placebo treatment group. To date, no serious or systemic adverse reactions have been reported. Thirteen veterinary-attended adverse events have been reported to date, of which seven were injection site reactions considered likely to be related to trial injection administration. No interactions between the test product vaccine or placebo and any concomitant treatments have been reported to date. The overall retention rate following randomisation and the first treatment administration is 87.9%. Three histologically confirmed EGS cases have occurred within the study population to date, representing an EGS incidence of 0.28 cases per 100 horse-years at risk.</p> <p>The first scheduled interim analysis was completed in April 2015. There were no significant differences between treatment groups for age ($p=0.28$), gender ($p=0.66$) or breed ($p=0.87$) distributions, indicating that the randomisation method used was successful in controlling for horse-level risk factors for EGS.</p> <p>Masking of treatment group allocations will be maintained until final data analyses are completed in early 2017, where the incidence of EGS will be compared between the vaccine and placebo treatment groups.</p>
<p>Have the results been published? If yes please state when:</p>
<p>No RCT results published to date. Results of preceding preliminary safety study and pilot field trial published as detailed in knowledge transfer section above.</p>