Summary of Project Aims and Progress

The aims of the project, commenced in October 2010, were to finalise EGS surveillance data gathering, produce detailed epidemiological analyses of the data and to make the necessary arrangements for planning a vaccine field trial.

Objective 1: To conduct a review of EGS surveillance in the UK for the period 2000-2009

Status: Completed

Just prior to the commencement of the current project, a peer reviewed publication was produced by the Animal Health Trust (AHT) Epidemiology and Disease Surveillance Group\(^1\) which provided a detailed description of epidemiological data for 1410 EGS cases occurring in Great Britain in the decade 2000-2009.

During the current project, further analysis of EGS surveillance scheme data was presented as an oral presentation at the 50\(^{th}\) British Equine Veterinary Association Congress\(^2\). This work used surveillance scheme data to calculate EGS incidence rates on affected premises and to investigate associations between the type of EGS and premises characteristics. A total of 1517 cases were reported from 1246 locations, of which 249 were 'recurrent' premises that either reported a history of previous cases or reported multiple cases to the surveillance scheme. The overall incidence was 1.95 cases per 100 horse years at risk, suggesting that one in every fifty horses on EGS-affected premises would develop the disease each year. EGS incidence rates did not differ significantly between individual countries in Great Britain, however, a significantly greater proportion of premises in Scotland had recurrent cases

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of EGS. Compared to premises reporting isolated cases, recurrent EGS premises were significantly larger in terms of premises size, with larger fields/paddocks and greater numbers of horses. Horses and ponies suffering from chronic EGS had been resident on the affected premises and paddocks for significantly longer periods compared to those diagnosed with acute or subacute EGS, which may be consistent with the natural acquisition of partial immunity.

These findings were also presented as a scientific poster presentation (Appendix 1), and will be submitted for publication in a peer-reviewed journal. Most importantly, these data have been used to identify eligible owners for participation in a feasibility study to inform the design of a randomised controlled vaccine field trial for a candidate vaccine against EGS. Moreover, calculated incidence rates have facilitated the identification of premises affected by a high frequency of EGS for inclusion in the vaccine field trial.

A collaborative project with the Department of Epidemiology & Population Health, University of Liverpool used advanced analytical techniques to investigate spatio-temporal distribution of EGS cases (i.e. geography and month/year of EGS occurrence) using data collected by the surveillance scheme. This is the largest study to date to explore space-time clustering of EGS, confirming the strong seasonality of EGS and identifying several high risk areas within Great Britain. Results of this study will be submitted for publication in a peer reviewed journal and presented wherever possible at appropriate conferences.

Data from the EGS surveillance scheme was also used as part of an Equine Science MSc student project with the Royal (Dick) School of Veterinary Studies, University of Edinburgh, comparing Equine Atypical Myopathy and EGS.

**Objective 2: To oversee the parallel testing of serum samples from the previous AHT vaccine safety trial and the Liverpool University epidemiological study.**

**Status: On-going**

Together with the Animal Health Trust’s immunology group, and in collaboration with Professor Ian Poxton, University of Edinburgh, we have secured funding to develop titration assays to measure the antibody response to *Clostridium botulinum* (*C. botulinum*) type C surface antigen and *C. botulinum* type C toxin complex toxoid (BoNT/C toxoid) in horses and ponies.

This project has been approved by the Animal Health Trust Clinical Research Ethics Committee and is due to commence in January 2013. Using these serological assays to quantify pre- and post-vaccination antibody titres to *C. botulinum* antigens will be essential in the evaluation of immune response to vaccination and also to further understanding of the relationship between *C. botulinum* type C and EGS. The tests could also have a future application in disease screening to inform an individual animal’s risk of developing EGS. For example, screening new animals moving on to EGS-affected premises could aid identification of those at increased risk of EGS. Preventive measures such as management changes, and potentially vaccination, can then be targeted towards these higher risk animals, playing an important role in EGS prevention. It is anticipated that at least one paper in a peer reviewed journal will be produced and results will also be presented at appropriate conferences.
**Objective 3:** To prepare and submit an application dossier to the Veterinary Medicines Directorate (VMD) for appropriate licensing of a *C. botulinum* Type C toxoid for use in a future UK-based vaccine field trial.

**Status: Completed**

A safety study of the candidate *C. botulinum* Type C toxoid vaccine has been undertaken (October 2011 – January 2012). In collaboration with the vaccine manufacturers, an application dossier for an Animal Test Certificate was submitted to the Veterinary Medicines Directorate (VMD) in June 2012. On 4th October 2012, the VMD issued an Animal Test Certificate approving the use of the candidate *C. botulinum* type C toxoid in a small pilot vaccine field trial. This pilot field vaccine trial has been granted ethical approval from the Animal Health Trust Clinical Research Ethics Committee and the University Of Edinburgh School Of Veterinary Medicine Ethical Review Committee. The pilot trial commenced on 19th October 2012, enrolling 95 client-owned horses and ponies, and will be completed in July 2013. Information from the pilot study will be used in the preparation of an application dossier for a further certificate for conducting a full nationwide randomised controlled vaccine trial. Furthermore, results from the pilot study will be used maximise efficiency of recruitment of both veterinary practices and horse owners for the subsequent nationwide full vaccine trial. The pilot study will also aid identification of potential issues in the conduct of the full vaccine trial and will inform modifications to the proposed study design and facilitate estimation of retention rates, which will improve the accuracy of sample size calculations.

**Objective 4:** To prepare a study justification and protocol for the conduct of a UK-based vaccine field trial

**Status: Study protocol completed**

A detailed study justification and protocol for the pilot field vaccine trial were submitted as part of the application dossier for the Animal Test Certificate to the VMD. Additionally, specifically designed owner and veterinary information packs have been produced for the pilot trial. These existing documents will be revised in accordance with findings resulting from the pilot trial for use in the subsequent nationwide full vaccine trial.

The process of securing the considerable funding required for the conduct of the full vaccine field trial has been initiated, with funds being sought from a range of sources, including charitable trusts, scientific research bodies and private benefactors.
Appendix 1: EGS surveillance scheme poster presented at SVEPM 2012

Epidemiology and Surveillance of Equine Grass Sickness in Great Britain

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Introduction
Equine grass sickness (EGS) is a predominantly fatal neurodegenerative disease affecting grazing horses, ponies and donkeys. Great Britain has the highest reported incidence of the disease worldwide.

Aims
To describe incidence rates and epidemiological aspects of EGS on British premises affected since 2000.

Methods
A nationwide surveillance scheme using postal and online questionnaires collected retrospective premises-level and prospective case-level information for EGS cases occurring in Great Britain between January 2000 and January 2011. Incidence rates were estimated from the number of horses on affected premises and time at risk from the start of the study period or from the date from which the premises history was known, if after January 2000. Recurrent premises were defined as those with a history of previous cases or where multiple cases had been reported to the surveillance scheme.

Results
A total of 1517 EGS cases were reported from 1246 locations in Great Britain since 2000. Complete location data was available for 1049 cases, of which:
• 66.9% occurred in England
• 36.4% occurred in Scotland
• 2.2% occurred in Wales

A mean of 138 cases per year were reported to the EGS surveillance scheme (range 75–233 cases per year), with peaks seen consistently in the spring (April – June; Figure 1).

![Histogram of the temporal distribution of 1517 EGS cases reported to the EGS surveillance scheme between January 2000 and January 2011.](image)

Figure 1: Histogram of the temporal distribution of 1517 EGS cases reported to the EGS surveillance scheme between January 2000 and January 2011.

Incidence of EGS
• Overall incidence was 1.95 cases of EGS per 100 horse years at risk.

<table>
<thead>
<tr>
<th>Feature of Premises</th>
<th>Median Incidence Rate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent premises</td>
<td>2.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Non-recurrent premises</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Scotland</td>
<td>2.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Wales</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Median incidence rates of EGS (cases per 100 horse years at risk) reported to the EGS surveillance scheme between January 2000 and January 2011. Mann Whitney U and Kruskal-Wallis p values, respectively, for difference in median incidence rate.

Recurrent Premises
A total of 249 locations were defined as "recurrent" EGS premises.

There was a significant association between country and recurrent premises (p<0.001).

Proportion of affected premises reporting recurrent EGS cases:
• Scotland 57.5%
• England 43.0%
• Wales 33.3%

Compared to non-recurrent EGS premises, recurrent EGS premises had significantly larger total premises size, larger size of affected paddocks, greater total number of horses and greater number of horses grazing affected paddocks.

Clinical Presentations
Clinical presentation was recorded for 1324 EGS cases, of which:
• 46.6% were reported as acute ([duration of clinical signs <3 days]
• 20.4% were reported as subacute ([duration of clinical signs 2–7 days]
• 33.0% were reported as chronic ([duration of clinical signs >7 days]

<table>
<thead>
<tr>
<th>Time on Premises</th>
<th>Median Acute/Subacute EGS Cases</th>
<th>Median Chronic EGS Cases</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time resident on affected premises</td>
<td>365 days</td>
<td>730 days</td>
<td>0.05</td>
</tr>
<tr>
<td>Time grazing affected paddock</td>
<td>60 days</td>
<td>90 days</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Table 2: Median time spent on affected premises and pasture of 1324 acute/subacute and chronic cases of EGS reported to the EGS surveillance scheme between January 2000 and January 2011. Mann Whitney U p value for difference in median values.

Conclusions
A greater proportion of recurrent premises were located in Scotland and were larger establishments. Horses with chronic EGS had been kept on affected premises and paddocks for longer periods prior to disease compared to acute and subacute cases, which could be consistent with some degree of acquired immunity or increased exposure to protective factors resulting in decreased severity of clinical signs. This information, particularly data regarding areas of increased disease incidence and high risk premises, will be valuable in the development of future intervention studies, such as vaccination field trials.

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