Pilot field vaccine trial for Equine Grass Sickness

Purpose of the pilot field vaccine trial

The main purpose of the pilot field vaccine trial was to gather information about the feasibility of performing a larger vaccine trial on a national scale in Great Britain. The pilot trial was therefore designed to test the methods used in the vaccine trial, rather than to test the vaccine.

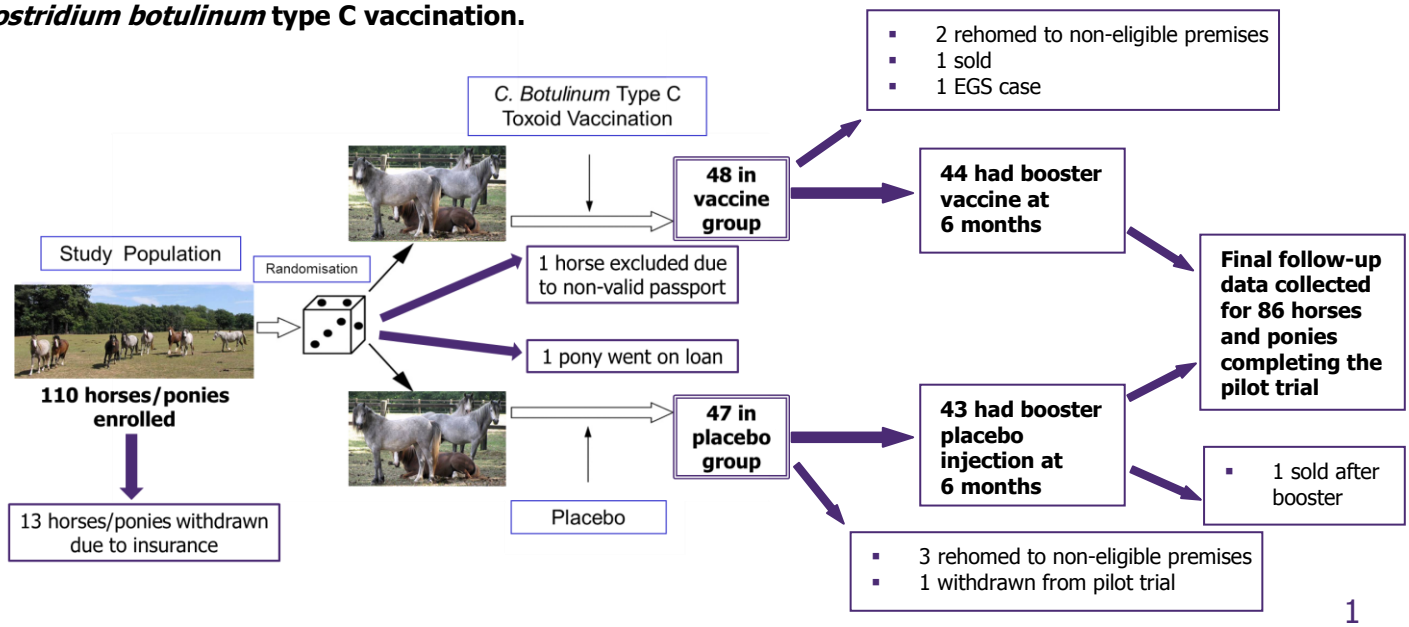
The pilot trial focussed specifically on:

- identifying the best way of recruiting veterinary practices and horse or pony owners interested in taking part in an EGS vaccine trial.
- identifying the most effective way of co-ordinating vaccination of all enrolled horses and ponies within a short time period.
- informing the most effective methods for gathering information about the health and management of all enrolled horses and ponies at regular intervals throughout the trial.
- measuring the immune response to vaccination with the *Clostridium botulinum* type C toxoid vaccine.

Methods used in the pilot field vaccine trial

The pilot trial was co-ordinated by the Animal Health Trust (AHT), in collaboration with the Royal (Dick) School of Veterinary Studies, University of Edinburgh. Five participating veterinary practices recruited ten eligible premises in Scotland, previously affected by a high number and frequency of Equine Grass Sickness (EGS) cases. A total of 95 horses and ponies residing on these premises were enrolled and on each individual premise, horses/ponies were randomly allocated to one of two approximately equally sized groups, using computer-generated random numbers – one group received a full vaccination course of the *Clostridium botulinum* type C toxoid vaccine and the other group received a full course of inactive placebo injections (Figure 1). The toxoid vaccine contains inactivated *Clostridium botulinum* type C toxins and this type of vaccine is very similar to the tetanus toxoid vaccine commonly used to prevent tetanus in horses and ponies. The placebo injection was selected based on similarities in both formulation and appearance to the vaccine. Neither participating owners nor veterinary surgeons knew which group each horse or pony was in during the trial – this is known as "blinding" or "masking", which is a method used routinely in good quality clinical trials.

Figure 1: Enrolment, group allocation and treatment administration for the pilot field trial of a *Clostridium botulinum* type C vaccination.



A total of 48 enrolled horses/ponies were randomly assigned to the vaccine group and 47 were assigned to the placebo treatment group (Figure 1), all of which completed the primary treatment course of three injections given 21 days part. Throughout the pilot trial, owners provided information via telephone questionnaires, and recorded details of preventive healthcare treatments, including wormers and vaccinations, administered for each enrolled horse/pony during the trial.

Prior to each injection, all enrolled horses/ponies received a thorough veterinary clinical examination, and findings were recorded using standardised protocols and pre-treatment clinical examination forms. Participating owners undertook daily post-treatment observations for a seven day period following each injection. This included thorough inspection of the injection site for signs of vaccination reactions together with assessment of appetite and demeanour, and findings were recorded on standardised recording forms.

Results of the pilot field vaccine trial

The pilot field vaccine trial was a huge success and all of the study objectives were met. Findings of the study have already been used to revise sample size calculations and trial methodology for the full-scale nationwide randomised placebo-controlled field vaccine trial. We have provided a summary of the findings of this study to the Equine Insurance Forum, which we hope will reduce any problems regarding insurance cover in recruitment for the forthcoming nationwide field vaccine trial.

Baseline premises and individual horse telephone questionnaires were useful in assessing inclusion criteria for enrolled horses and ponies, in addition to gathering information regarding risk factors for EGS. The average age of enrolled horses and ponies at the start of the pilot study was 6 years (ranging from 6 months to 23 years).

Both the *C. botulinum* type C toxoid vaccine and placebo injection were shown to be safe. No systemic adverse reactions (where the entire body may be affected and the horse or pony may become unwell) were reported following any injections administered during the pilot vaccine trial. Overall, 19 minor local injection site abnormalities (such as localised heat and/or pain and/or swelling at the injection site) were reported, following a total of 372 injections administered during the study, none of which required treatment or veterinary attention. There was no significant difference in the number of minor injection site abnormalities between the vaccine or placebo treatment groups.

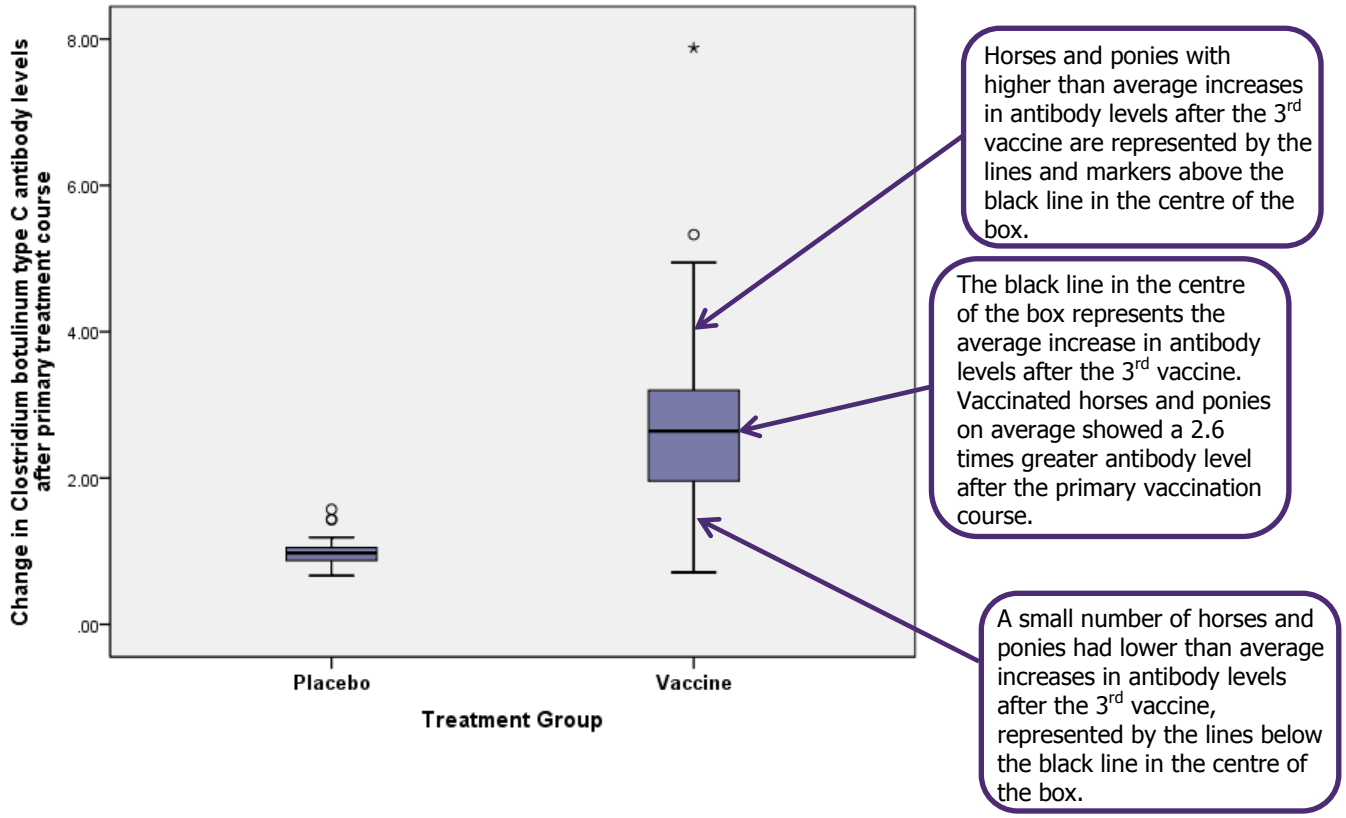
Immune response to vaccination

Using a blood test developed by the Animal Health Trust's Immunology Group, we were able to demonstrate significant increases in antibody levels following vaccination. The placebo injection had the same formulation as the vaccine except that did not contain the active component (the inactivated *C. botulinum* type C toxins), and as expected, horses and ponies in the placebo treatment group did not have a significant increase in their antibody levels following completion of the primary course. In contrast, horses and ponies in the vaccine treatment group showed an increase in antibody levels following the primary vaccination course, with the antibody levels after the third vaccine on average 2.6 times higher than the antibody levels before the first vaccination. Fifty percent of horses and ponies in the vaccine treatment group had an increase in antibody levels following the third vaccine of between 2 and 3.2 times their pre-vaccination antibody levels. This demonstrates that the majority of horses and ponies in the vaccine treatment group had a significant immune response following the primary vaccination course, which is shown in Figure 2.

In Figure 2, all the values for the change in antibody levels for horses and ponies in the placebo group are clustered just above zero, with a very limited range of values. This would be expected as the placebo injection would not trigger an immune response or provide any form of immunity. In contrast, the majority of horses and ponies in the vaccine treatment group had changes in antibody levels well above zero, indicating an immune response to vaccination. However, the range of values in the vaccine treatment group is much wider, which reflects the large variation in individual immune response following the primary vaccine course. Some horses/ponies have a very strong immune response following the primary course, such as the horse/pony marked with the asterisk in Figure 2, whose antibody levels increased nearly 8 times after the third vaccine. Conversely, some other horses and ponies have a lower immune response to vaccination, showing an increase in antibodies of 1 to 2 times their pre-vaccination level (shown by the lower vertical line in Figure 2). This is a very similar pattern to that which is seen following other equine vaccinations, including the widely used equine influenza vaccines. Factors which are known to influence an individual horse or pony's response to vaccination include age, worm burden, having a disease at the time of vaccination and administration of some other medications around the time of vaccination. It is for these reasons that in the full-scale nationwide vaccine trial, we would expect to see EGS cases occurring in both the vaccine and the

placebo treatment groups. If the *C. botulinum* type C toxoid vaccine is effective in reducing the risk of EGS, we would expect to see significantly fewer cases in the vaccinated group compared to the group which receive the inactive placebo.

Figure 2: Analysis of *Clostridium botulinum* type C antibodies in the vaccine and placebo treatment groups. This figure shows the change in antibody levels between the blood samples taken before the first injection and those taken 2 – 4 weeks after the third injection in both treatment groups.



Incidence of EGS

One confirmed case of EGS occurred during the pilot vaccine trial, affecting a horse in the vaccine treatment group, and a further three cases occurred on a participating premise in unvaccinated horses/ponies not enrolled in the study. Using the blood test described above, it was shown that the vaccinated horse affected by EGS did not mount a significant immune response following the primary vaccination course, exhibiting only a 1.2 times increase in antibody levels (a similar change to that seen in some horses and ponies in the placebo treatment group).

Conclusions

This pilot study has provided invaluable information which has been used to improve methods used in the recruitment and monitoring phases for the forthcoming full-scale nationwide EGS vaccine trial. The recruitment methods used were successful in identifying high risk EGS-affected premises, with 95 eligible horses/ponies enrolled within six weeks. Co-ordination of treatment administrations was straightforward, and participating owners and veterinary surgeons did not report any difficulties in undertaking the scheduled field visits.

No systemic adverse reactions were reported, and the incidence of local injection site abnormalities was low, providing evidence of vaccine safety under conditions of field use. No interactions between the vaccine or placebo and any other treatments were reported throughout the study period.

Blood tests showed that horses and ponies in the vaccinated group had a significantly greater increase in antibody levels following the primary treatment course compared to those in the placebo treatment group, indicating immune response to primary vaccination.