Equine grass sickness (EGS) is a polynuropathy with a case mortality rate of approximately 85%. While epidemiological studies have identified numerous risk factors, there is currently no preventive healthcare measure available for EGS prevention. Previous research suggests that EGS may represent a toxico-infectious form of botulism involving Clostridium botulinum type C. Other equine clostridial diseases are successfully prevented by vaccination, implying that it should be possible to prevent EGS by vaccination. As EGS cannot be induced experimentally, a field trial represents the only method of evaluating the effect of vaccination. This pilot study aimed to inform sample size and trial methodology for a proposed full-scale triple-blinded randomised placebo-controlled field trial of a C. botulinum type C toxoid vaccine in the prevention of EGS. Healthy client-owned horses/ponies residing on premises previously affected by a high incidence and frequency of EGS were included. Horses/ponies were randomly allocated, at premises-level, stratified by age, to vaccine/placebo groups at a 1:1 ratio. Baseline and follow-up premises and horse data were obtained via telephone questionnaires. Participating veterinary surgeons administered the primary course of vaccine/placebo on days 0, 21, 42, followed by a booster injection on day 224. Following appropriate training, owners completed post-treatment daily observations for seven days following each treatment. Five participating practices recruited 10 EGS-affected premises in Scotland, with a median baseline incidence of 2.2 EGS cases per 100 horse-years-at-risk. 109 horses/ponies were enrolled: 13 were withdrawn prior to randomisation, and 1 was excluded following randomisation (Figure 1). Median age at enrolment was 6 years. Age (p=0.34), gender (p=0.15) and breed (p=0.94) distributions did not differ between treatment groups. 95 horses/ponies completed the primary treatment course, and 87 horses/ponies received booster injections, representing a retention rate of 90.5%. No significant adverse events were reported and incidence of owner-reported injection site abnormalities was 0.05 per horse-week-at-risk, none of which required treatment. One histologically-confirmed EGS case occurred during the study, representing an incidence of 1.25 cases per 100 horse-years-at-risk. There was no difference in the risk of EGS between treatment groups (p=0.62). Serological analyses demonstrated a significant increase in C. botulinum type C antibody titres following the primary treatment course in vaccinated horses/ponies compared to the placebo group (p<0.001), indicating seroconversion following primary vaccination. This study provided evidence of vaccine safety under conditions of field use, serological evidence of immune response to vaccination and informed modifications to trial methodology for a subsequent nationwide field trial. This study was funded by Neogen Corporation and the Animal Health Trust.

Figure 1. Enrolment and treatment group allocation in a pilot field trial of Clostridium botulinum type C vaccination for EGS prevention.