047 Vaccination against Equine Grass Sickness: piloting a clinical field trial of *Clostridium botulinum* type C toxoid in the United Kingdom


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Equine grass sickness (EGS) is a polyneuropathy 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 control trial. This pilot study aimed to inform sample size and trial methodology for a proposed full-scale triple-blinded randomised control trial.

109 horses/ponies were enrolled: 13 were withdrawn prior to primary vaccination, 95 horses/ponies completed primary treatment course, and 87 horses/ponies received booster injections, representing a retention rate of 90.5%. No armed EGS case occurred during the study, representing a retention rate of 90.5%. No armed EGS case occurred during the study, representing a retention rate of 90.5%. No armed EGS case occurred during the study, representing a retention rate of 90.5%. No armed EGS case occurred during the study, representing a retention rate of 90.5%. No armed EGS case occurred during the study, representing a retention rate of 90.5%. No armed EGS case occurred during the study, representing a retention rate of 90.5%.


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with a median baseline incidence of 2.2 EGS cases per 100 horse-years-at-risk. 109 horses/ponies were enrolled: 13 were withdrawn prior to randomisation, and 1 was excluded following randomisation (Figure 1).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.

142 Peptide motifs for Major Histocompatibility Complex class I molecules of the horse


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Knowledge of the anchor motifs of equine Major Histocompatibility Complex (MHC) class I molecules and identification of the pathogen-derived peptides that bind to them is critical for the design of vaccines that can stimulate effective T-cell mediated immunity to prevent diseases caused by Equine Influenza Virus, Equine Herpesvirus, and other microorganisms. Recently we determined the peptide binding motif of the Equine Leukocyte Antigen ELA-A3.1 classical MHC class I molecule using a standard immunological approach (Bergmann et al., 2015). Endogenously bound peptides were eluted from P-815 cells transfected with the ELA-A3.1 gene using an equine specific monoclonal antibody. The peptides were sequenced using tandem mass spectrometry and revealed a nonamer with anchoring amino acids at positions 2 (aspartic acid) and 9 (isoleucine or leucine). Using an independent method horse MHC class I expression was stabilized in RMA-S cells transfected with ELA-A3.1 by screening with a library of 15-mer peptides that overlapped by 10 amino acids and spanned the entire Intermediate Early (IE) protein of Equine Herpesvirus type 1 (EHV-1). One of the IE-derived peptides (PPARDGARFGELAAS) stabilized the ELA-A3.1 MHC class I molecule. This was confirmed and the sequence further narrowed by testing with shorter peptides. The promiscuously binding nonamer SDYLELDT1 also stabilized ELA-A3.1 expression. Taken together, these results point to the EHV-1 IE-derived peptide RDGARFGEALS as a candidate for studies of cytotoxic T-cell immunity to EHV-1 in the horse. Other in vivo studies have shown that the IE protein contains immunogenic peptides that can induce protective immunity against this important viral pathogen. These results pave the way for development of assays for antigen-specific equine T-cells that can be used in studies of viral immunity or to assess the efficacy of vaccines.

Acknowledgements

This research was supported in part by a grant from the Harry M. Zweig Memorial Fund for Equine Research in New York State. DFA is an investigator of the Dorothy Russell Havermeyer Foundation.

References

Isolation and characterization of bacteriophages against equine pathogens—novel phages revealed as phage therapy candidates


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A role for bacteriophage therapy was envisaged early last century; however, due to discovery of the antimicrobials, it fell out of research interest. Currently, bacteriophages are resurfacing as an alternative to antimicrobials in order to overcome the increasing incidence of antimicrobial resistance. Here, we report isolation of bacteriophages against Escherichia coli, Shigella spp., Aeromonas hydrophila and Citrobacter sedlakii isolates of equine origin. Phages were isolated from equine farm soil and sewage samples. For enrichment, sample aliquot was incubated overnight with host bacteria at 37°C with vigorous shaking. The crude lysate obtained was centrifuged and filtered and the presence of any phage in the suspension was detected by agar overlay technique. Appropriate dilutions of enriched samples were plated to obtain individual plaques and the most dominant plaque was transferred into SM buffer, serially diluted and plated for plaque re-isolation three times to ensure purity, followed by large scale preparation of phage stocks. Any host nucleic acids was degraded using pancreatic DNaseI and RNase and bacteriophage particles were precipitated using PEG8000. Phage titre was determined by plaque assay and phage concentrates were accessioned in the Veterinary Type Culture Collection (VTCC) repository. The phage concentrates were visualized by transmission electron microscopy (TEM). The temperature stability of bacteriophages was checked after incubating phage concentrates over the range of 4°C–80°C temperature for one hour. A clear single plaque was obtained on nutrient agar against Shigella spp. and after purification and concentration, its analysis by electron microscopy revealed presence of multiple phages belonging to families Myoviridae, Siphoviridae and Podoviridae. However in case of Citrobacter sedlakii, a Siphoviridae phage (VTCCBPA61) with dimensions: 60 nm x 650 nm was observed. Against a pathogenic A. hydrophila isolate of equine origin (expressing aerolysin gene), a Myoviridae phage (VTCCBPA6) was isolated with dimensions: 62 nm x 138 nm. Against E. coli of equine origin, a Myoviridae phage (VTCCBPA9) of dimensions: 86 nm x 138 nm was observed. Bacteriophage VTCCBPA61 against C. sedlakii was found to completely lose its biological activity at 65°C in vitro however the group of phages against Shigella spp. were found to be stable upto temperature as high as 80°C. Thus we demonstrated the basic biological characteristics of phages, and some novel ones (such as against A. hydrophila and C. sedlakii) bacterial isolates of equine origin which have never been reported till now. These lytic phages could find potential in phage therapy, as biocides, in biosensors and in phage ligand technology and are being explored by us further to depict their therapeutic value in small animal model. As more studies are reporting safety, tolerance and efficacy of phage therapy in humans and animals, their use in phage therapy has a promising future as an emerging alternative to chemical agents.