pregnancy loss in Thoroughbred mares and a specific MHC class I allele in the mother. This association requires independent validation and further investigation of the mechanism by which the mare's genetic background contributes to pregnancy outcome.

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Development of recombinant VHH nanobodies as an alternative for clearance of equine arteritis virus in carrier stallions

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Equine viral arteritis (EVA) is a respiratory and reproductive disease of widespread occurrence among equid populations. EAV can cause long-term persistent infection in 10-70% of the infected stallions (carrier state), and the establishment and maintenance of EAV carrier state is testosterone-dependent. Carrier stallions constitute the natural reservoir of EAV and they continue to shed virus in semen for a long period of time ranging from weeks to life-long., EVA has a significant economic impact on the equine breeding industry due to the restriction of international movement of horses and the use of carrier stallions for natural and artificial breeding. Several therapeutic approaches to clear the carrier state (gonadotropin releasing hormone [GnRH] antagonists, anti-GnRH vaccination, antiviral compounds) or eliminate semen infectivity, (single-layer centrifugation) have been evaluated. However, none of them guaranteed clearance of persistent infection or elimination of virus infectivity from semen. Consequently, there are currently still no means available to fully eliminate the carrier state other than castration, which implicates the loss of the commercial value of the stallion. In the 1990's, heavy chain antibodies (HCAbs) were characterized in members of the *Camelidae* family. HCAbs are devoid of both the light chain and a constant heavy domain (CH1), and contain an antigen-specific variable heavy chain domain (VHH). This single VHH domain is also known as Nanobody (Nb). Several beneficial properties (size, affinity, stability, target accessibility) in combination with easy and low production costs has lead to their success as potential therapeutic agents for a variety of human and animal diseases. The objective of this study was to develop EAV-specific neutralizing Nbs as a potential therapy to induce viral clearance in carrier stallions. A single llama was immunized on days 0, 14, 28, and 57 with 1.5 ml of the modified live virus vaccine strain (ARVAC®) and the Argentina 2010 field strain of EAV ($10^{7.5}$ $TCID_{50}$/ml). Seroconversion was demonstrated following the OIE (World Organisation for Animal Health)-prescribed virus neutralization test (VNT; titer 1:6,400). Four days after the final boost, peripheral blood mononuclear cells were isolated from the buffy coat fraction by gradient centrifugation, lysed, and total RNA was obtained by phenol/chloroform extraction. The cDNA was synthesized using oligo(dT) primers, followed by a two-step nested polymerase chain reaction (nPCR) to amplify the VHH repertoire. The primers used for the first amplification reaction (CALL001 and CALL002) specifically anneal to constant and variable domain sequences conserved among all IgG isotypes. PCR fragments of 700bp corresponding with HCAb sequences were gel purified, and subjected to a second PCR reaction using primers, amplifying specifically the VHH repertoire. The final PCR products were cloned into the pMECS vector and transformed TG1 electrocompetent *E.coli*. A library of $3.3x10^7$ transformants was generated, with 71% containing the correct insert size as determined by colony PCR. Panning of the VHH repertoire by phage display using a panel of recombinant EAV major structural proteins is currently ongoing. In conclusion, a VHH library derived from llama B cells following immunization with EAV was generated. This novel strategy could allow the generation of EAV-specific neutralizing Nbs as a therapeutic approach to induce clearance of EAV carrier state in stallions.

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Construction and manipulation of a full-length infectious bacterial artificial chromosome clone of equine herpesvirus type 3

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Equine herpesvirus type 3 (EHV-3) is the causal agent of equine coital exanthema, a disease characterized by pox-like lesions on the penis of stallions and the vulva of mares. Although the complete genomic sequence of EHV-3 has been recently made available, its genomic content remains poorly characterized and the molecular mechanisms of disease development not yet elucidated. In an attempt to facilitate the genetic manipulation of EHV-3, we describe here the construction of a full-length infectious bacterial artificial chromosome (BAC) clone of EHV-3. Mini-F vector sequences were inserted into the intergenic region between ORF19 and ORF20 (the UL41 and UL40 homologs, respectively) of EHV-3 strain C175 by homologous recombination in equine dermis cells (Nbl-6). DNA of the resulting recombinant virus was electroporated into E. coli and a full-length EHV-3 BAC clone was recovered. The reconstituted virus obtained after transfection of the EHV-3 BAC into Nbl-6 cells showed growth properties *in vitro* that were indistinguishable from those of the parental virus. To assess the feasibility of mutagenesis of the cloned EHV-3 genome, a mutant virus with a deletion of the glycoprotein E gene was generated using the Red recombination system in E. coli and its *in vitro* growth properties evaluated. The cloning of EHV-3 as a BAC will simplify future studies to identify the role of its coding genes in viral pathogenesis and host immune response.

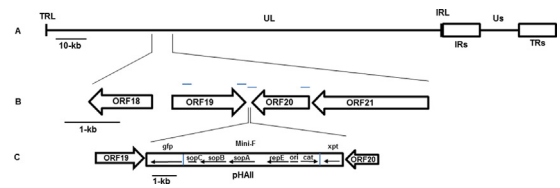

Figure. Strategy for generation of the EHV-3 BAC plasmid. (A) The structure of the EHV-3 genome with the UL and US components is shown at the top. Terminal and internal repeat sequences are indicated (B) Genomic organization from ORF18 to ORF21 of the UL region of the EHV-3 genome. Representative positions of the primers used for amplification and subsequent cloning of homologous arms are shown. (C) The mini-F vector (enclosing sequences for F-plasmid replication and the cat gene), the eukaryotic selection marker xgpt and the gfp gene were integrated between the ORF19 and ORF 20 ORFs in the UL region of the EHV-3 viral genome by homologous recombination in Nbl-6 cells. A full-length EHV-3 BAC clone was recovered after electroporation in E. coli.