Evolution of PRRSV in an Experimentally Infected Swine Herd

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Abstract

In 2005, 109 pigs were inoculated with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) strain VR-2332 and housed together with 60 control pigs for 203 days as part of The Big Pig Project, a multi-institutional collaborative effort to simulate and study PRRSV persistence in a typical swine herd. At various timepoints throughout the study, pigs were randomly selected and necropsied to create a bank of tissue and serum samples for use in future studies. During the course of The Big Pig Project, three separate spikes in serum antibody levels were detected post-inoculation across the herd. Our goal is to determine whether new strains of PRRSV evolved in individual pigs and spread throughout the herd, resulting in the increased antibody levels, or if another mechanism is responsible for the trends. We will do this by cloning and sequencing three genes of interest from submandibular lymph node tissue samples obtained at 42, 56, and 203 days post-inoculation from The Big Pig Project. The genes that have been chosen for sequencing are nsp2, ORF5, and ORF6. Nsp2 codes for a polymerase/replicase polyprotein. Interest in this gene and its role in PRRSV pathogenesis and persistence has risen in recent years, as a mutation in this gene has been used as an epidemiologic marker for a severely pathogenic form of the virus discovered in China in 2006. ORF5, the second gene of interest, codes for an envelope protein that is believed to be a target for neutralizing antibody. ORF6, a matrix protein gene, is highly conserved across PRRSV species and will serve as a control to ensure that variation found in the other two genes will be representative of true genetic diversity and not result from errors in methodology.

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS), one of the most economically devastating infectious diseases of swine worldwide, is characterized by reproductive failure and respiratory disease in pigs. PRRS is caused by a single-stranded, enveloped RNA virus (PRRSV). As with other RNA viruses, PRRSV is marked by a rapid rate of mutation and exists as a "quasispecies" population within infected individuals. The virus can be transmitted through direct or indirect contact, and is known to persist subclinically in pigs for up to five or six months. Currently, no efficient vaccinations for PRRSV have been developed.

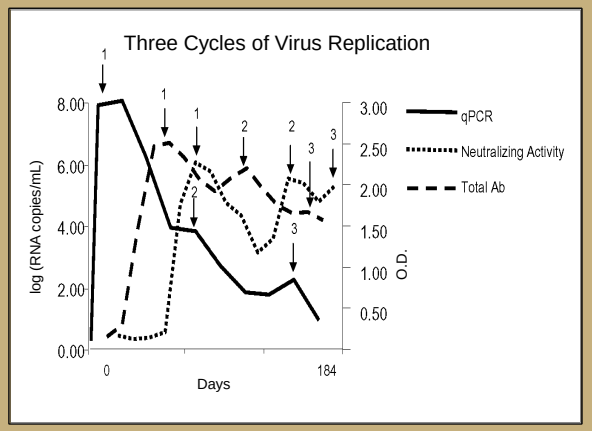


Figure 1: During The Big Pig Project, three separate spikes in serum antibody levels were detected across a herd of pigs post-inoculation with PRRSV.

The goal of this study is to determine if new strains of PRRSV evolved in individual pigs over time and spread throughout the herd, resulting in the separate increases in antibody levels.

Current Progress

To date, twelve submandibular lymph node samples have been screened for the presence of PRRSV RNA. Six have been found to be positive. Plasmids containing ORF5 from two of the above positive tissue samples have been cloned and sent for sequencing.

Methods

RNA extraction

Submandibular lymph node tissue samples collected at necropsy were stored in Ambion's (Foster City, CA) RNAlater solution. RNA was extracted from homogenized tissue using the Macherey-Nagel (Düren, Germany) NucleoSpin® RNA II kit.

PCR amplification

cDNA was synthesized from extracted RNA using the reverse PCR primer, with the Ivitrogen (Carlsbad, CA) SuperScript TM III First-Strand Synthesis System for RT-PCR. Two reactions per sample were prepared. cDNA was amplified using Invitrogen's Platinum® Pfx DNA polymerase with 35 PCR cycles of the following conditions: 30 seconds at 94˚C, 30 seconds at 55˚C, and 1 minute at 68˚C. Pools of cDNA, as well as a proof-reading Taq were used to minimize errors induced by the methodology. Primers from highly conserved regions of the PRRSV genome, specific for the open reading frame of interest were used.

Cloning

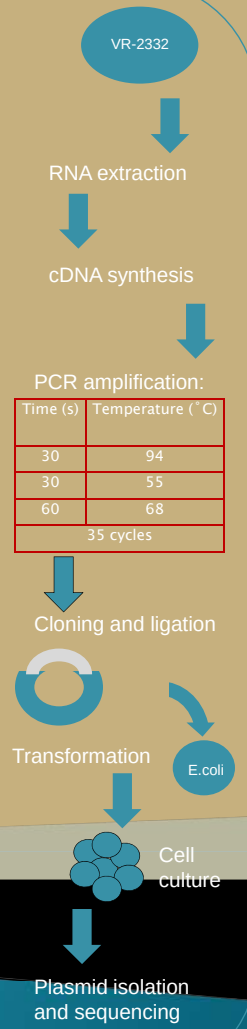
The PCR products were pooled and directly cloned. The Promega (Madison, WI) Wizard® SV Gel and PCR Clean-up System was used for DNA purification, as needed. Blunt ligation was performed with Invitrogen's Zero Blunt® TOPO® PCR Cloning kit. One Shot® TOP10 Chemically Competent E. coli cells were used for transformation. Terrific broth with kanamycin media was used for cell culture.

Plasmid purification and sequencing

The QIAprep Spin Miniprep kit from Qiagen (Venlo, Netherlands) was used for the isolation of plasmid DNA. Restriction digests were performed with EcoR1 enzyme to determine if the insert was present. Plasmids containing the appropriate insert were sent to the University of Missouri DNA Core for sequencing with M13 primers present on the plasmid in order to sequence the fragment in both directions.

Sequence analysis

Multiple clones from each sample and each open reading frame will be sequenced and analyzed using DNAStar to determine the quasispecies population present in different animals at different timepoints.



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