

Now You See Me, Now You Don't: Antigenic Variation and Replication Phenotype of Porcine Reproductive and Respiratory Syndrome Virus Variants

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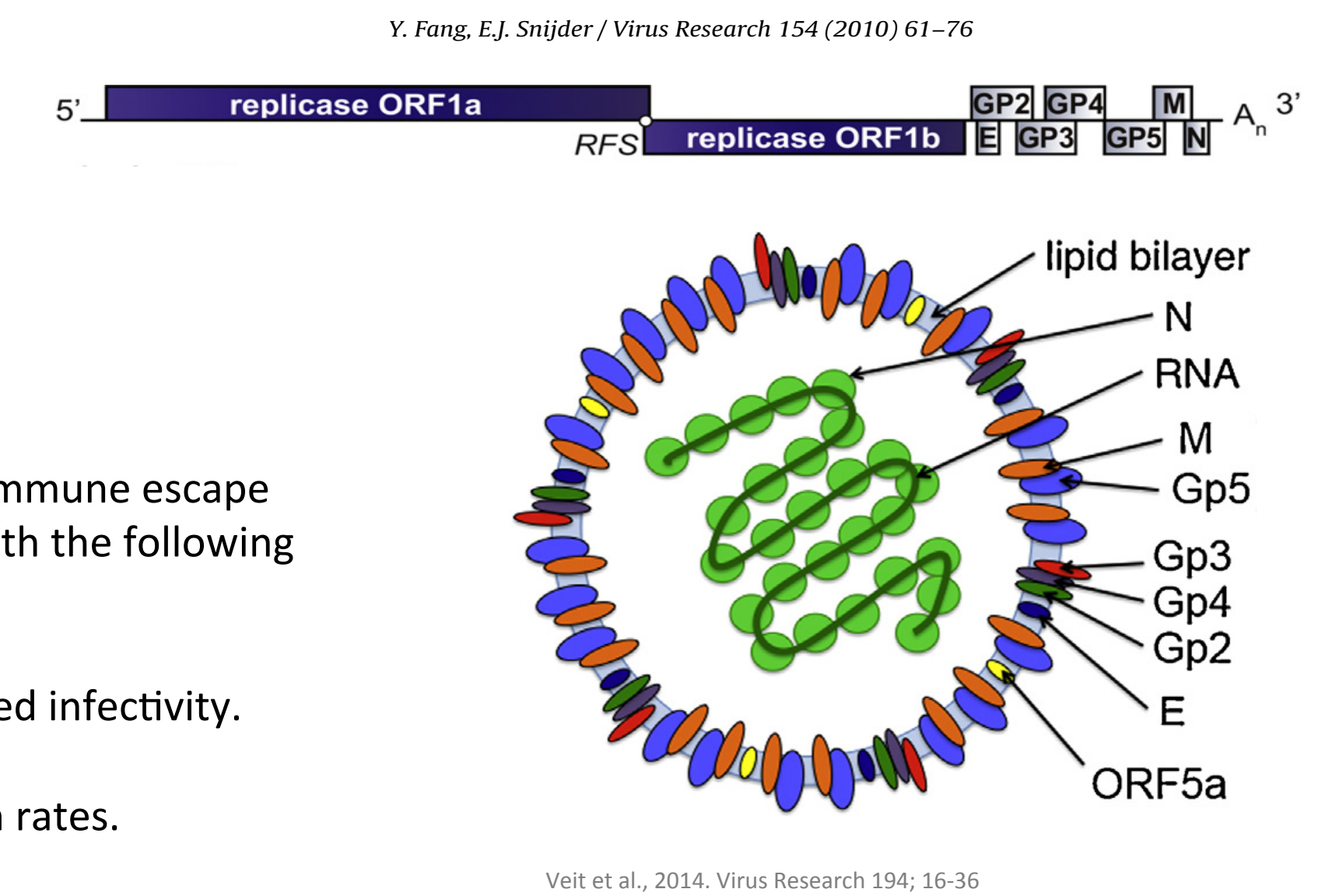
1. Abstract

RNA viruses are known for their genetic variability, allowing for rapid adaptation to changing host environments. Genetic and antigenic variation can confer viral escape from the host immune response but can often be associated with a decrease in viral fitness. Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus that causes an economically devastating disease of swine. In order to evaluate fitness costs associated with immune escape, we analyzed a panel of PRRSV variants for trade-offs in viral fitness. To determine if escape was accompanied by changes in replication fitness, the PRRSV variants were characterized for infectivity and growth kinetics in vitro. All four escape variants were significantly less infectious than both the parental FL12 virus and non-escape variants, indicating immune escape is associated with a decrease in infectivity. PRRSV variants differed in replication kinetics, but differences were not associated with an immune escape phenotype. Together, these results indicate that genetic changes in multiple PRRSV envelope proteins conferring immune escape are associated with decreased infectivity, and may alter replication rate.

2. Introduction and Objectives

RNA viruses are known for their genetic variability, allowing for rapid adaptation to changing host environments. Genetic and antigenic variation can confer viral escape from the host immune response but can often be associated with a decrease in viral fitness. Porcine reproductive and respiratory syndrome virus (PRRSV), in particular, is known for its variability and persistence in swine populations.

The PRRSV envelope proteins, encoded by ORF2-6, play a key role in virus attachment and entry into the host cell. Thus, they are a major target for host neutralizing antibody. Changes in the envelope proteins enabling escape from the host immune response have the possibility of affecting the virus's replicative fitness by reducing the capacity of attachment or entry.

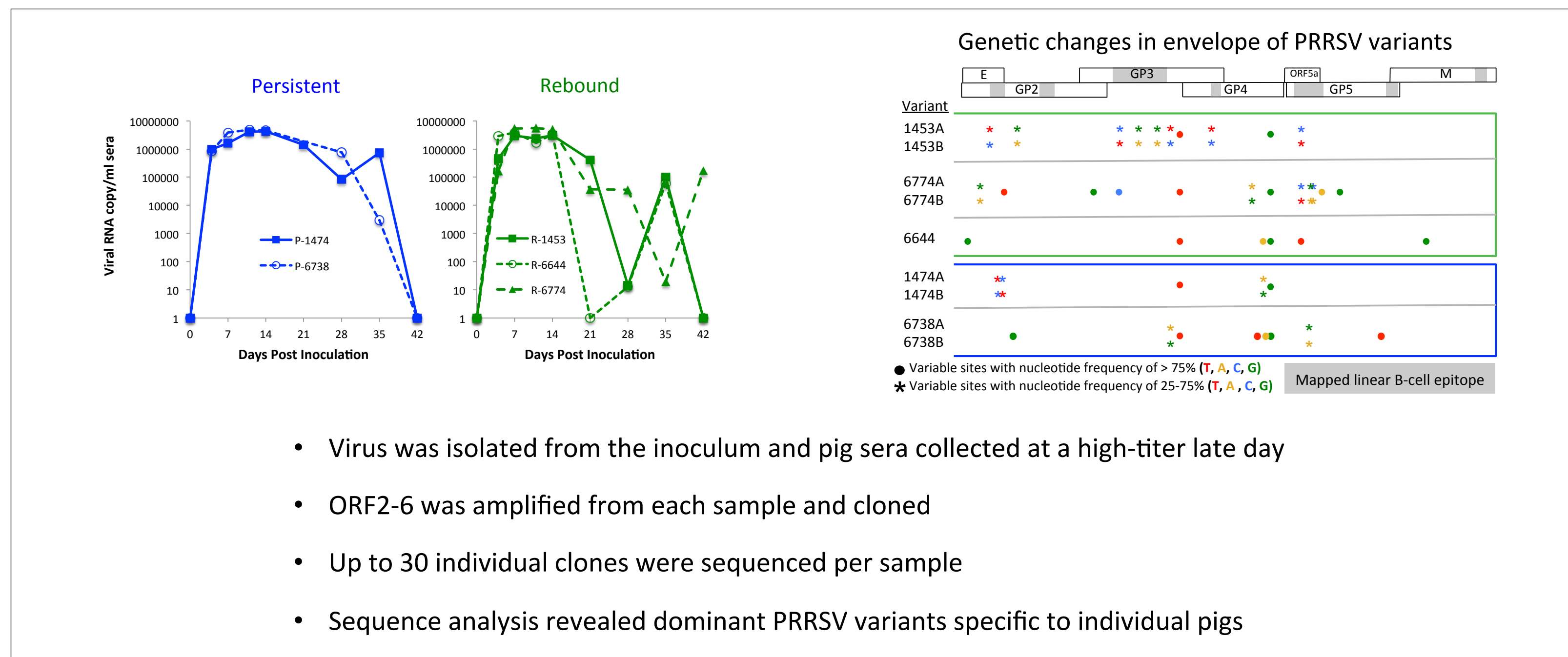


In the present study, we tested the hypothesis that PRRSV immune escape variants are associated with changes in replicative fitness with the following objectives:

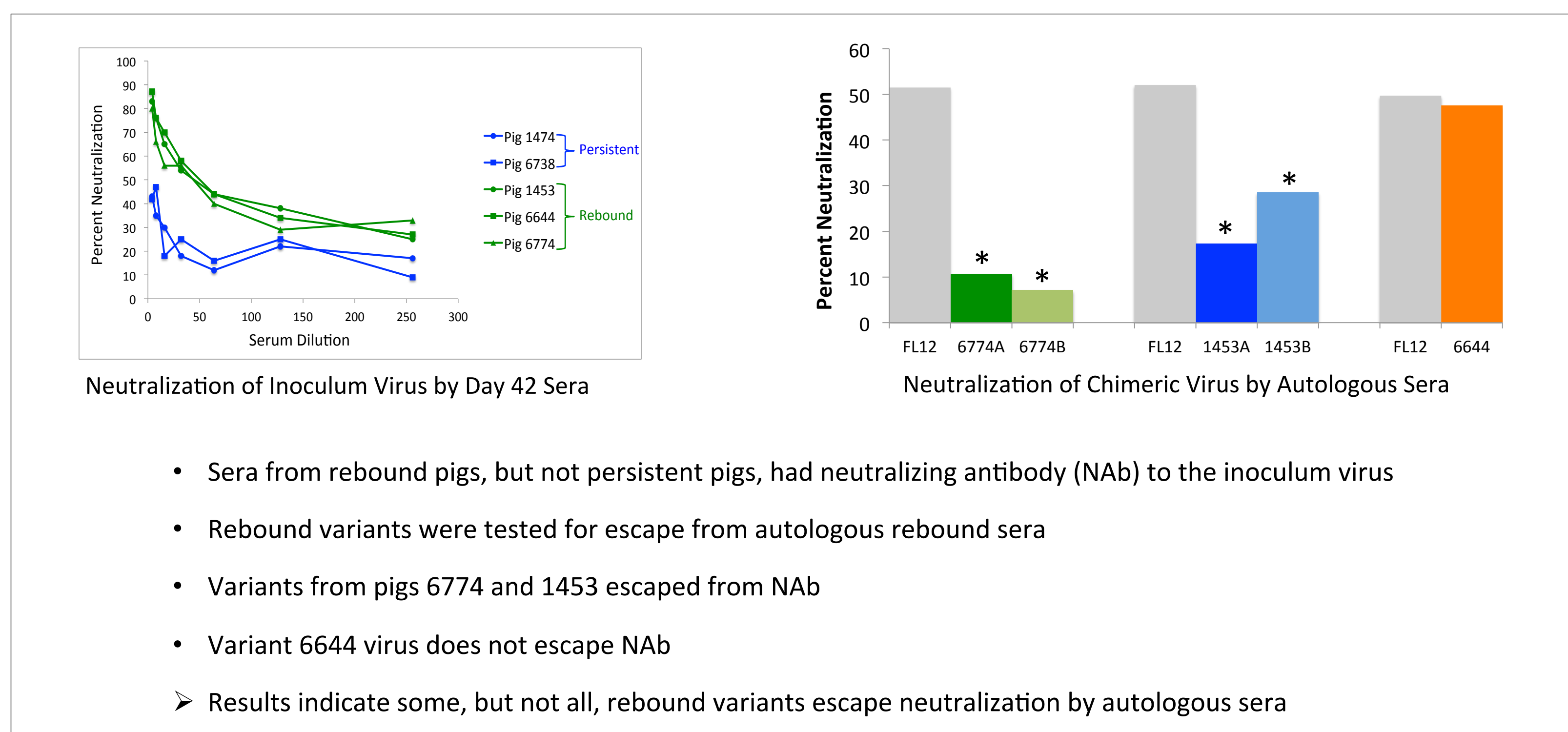
1. Determine if immune escape is associated with decreased infectivity.
2. Determine if PRRSV variants display different replication rates.

3. Genetic Variation and PRRSV Escape Variants

A recent study from the PRRS Host Genetics Consortium (PHGC) reported that up to 25% of experimentally infected pigs initially controlled virus replication but experienced a rebound in viremia by 42 dpi. Characterization of viral genotypes in five PHGC pigs that displayed differing virological outcome revealed distinct PRRSV variants, including immune escape variants.

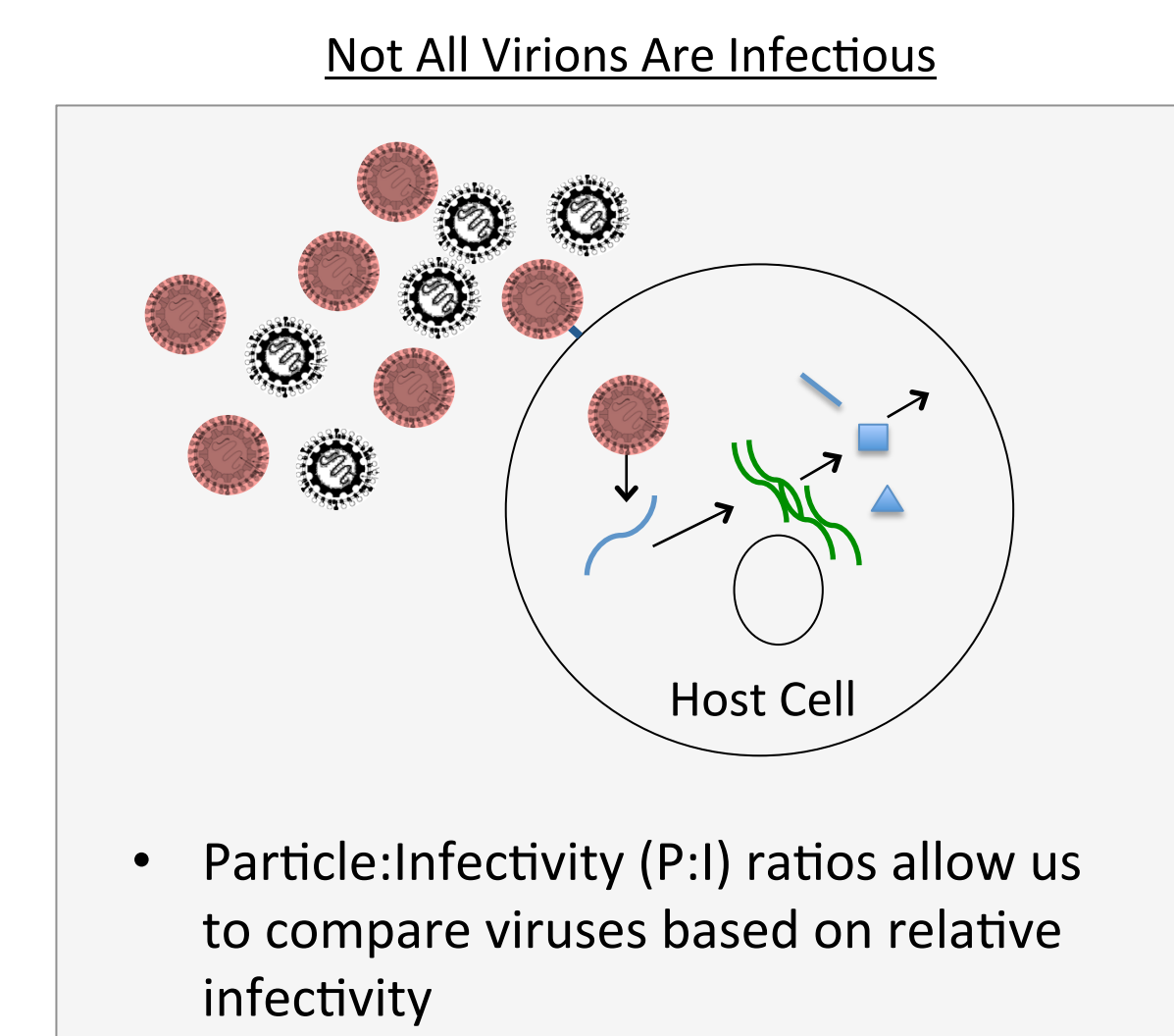


- Virus was isolated from the inoculum and pig sera collected at a high-titer late day
- ORF2-6 was amplified from each sample and cloned
- Up to 30 individual clones were sequenced per sample
- Sequence analysis revealed dominant PRRSV variants specific to individual pigs

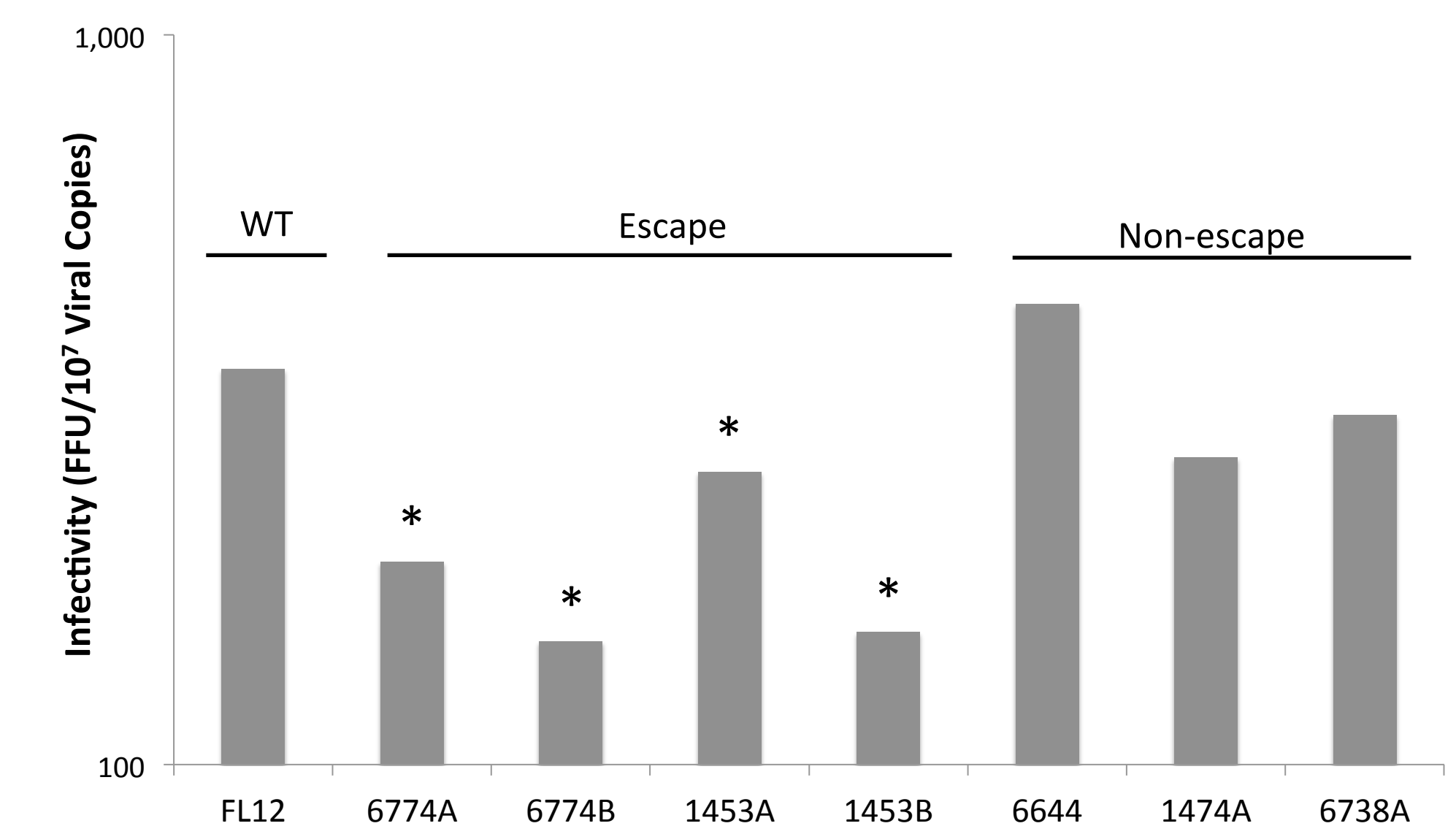
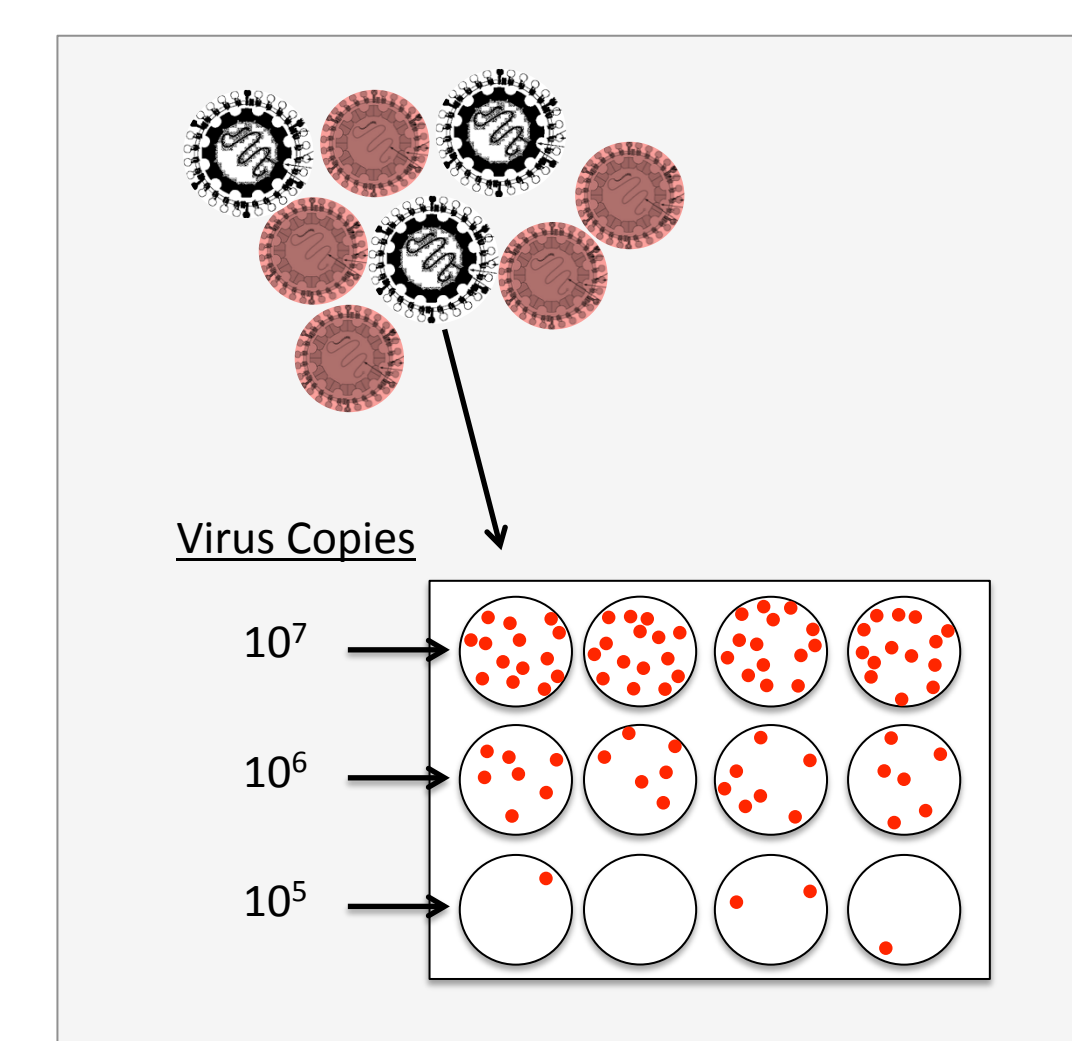


- Sera from rebound pigs, but not persistent pigs, had neutralizing antibody (NAb) to the inoculum virus
- Rebound variants were tested for escape from autologous rebound sera
- Variants from pigs 6774 and 1453 escaped from NAB
- Variant 6644 virus does not escape NAB
- Results indicate some, but not all, rebound variants escape neutralization by autologous sera

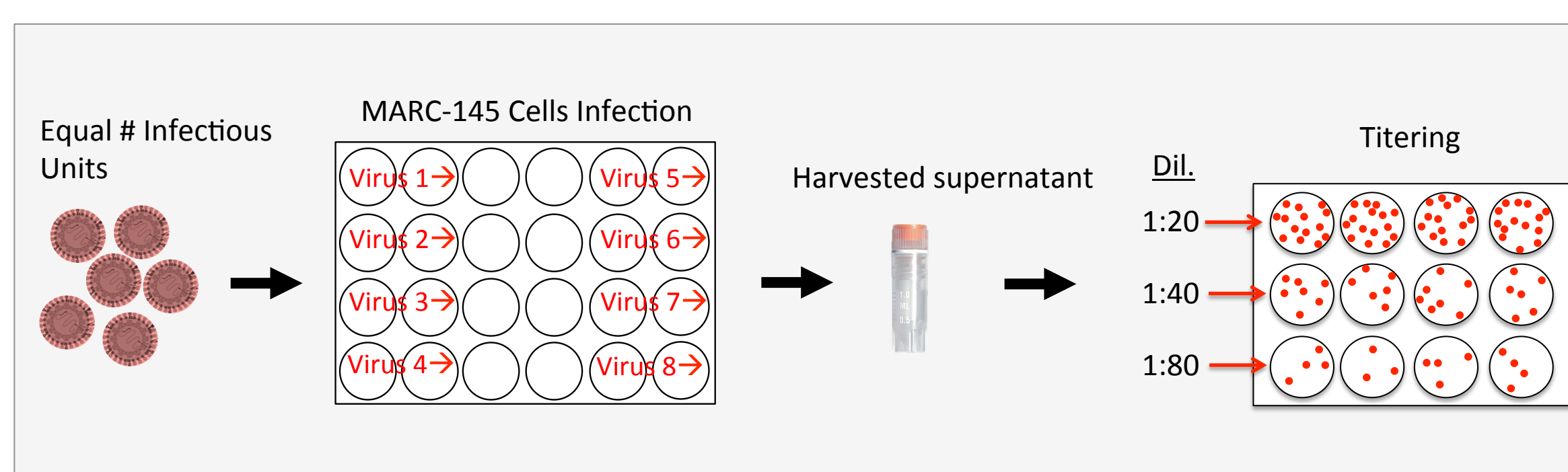
4. Immune Escape is Associated with Decreased Infectivity



- Infectivity of variants was determined by calculating particle to infectivity (P:I) ratios
- P:I ratios were determined by infecting MARC-145 cells with equal number of virus copies for each PRRSV variant
- PRRSV variants from pigs 6774 and 1453 were less infectious than FL12
- The variants from rebound pig 6644 and persistent pigs 1474 and 6738 had similar P:I ratios as FL12
- The four neutralizing antibody escape variants are less infectious than FL12, suggesting a loss of infectivity associated with immune escape

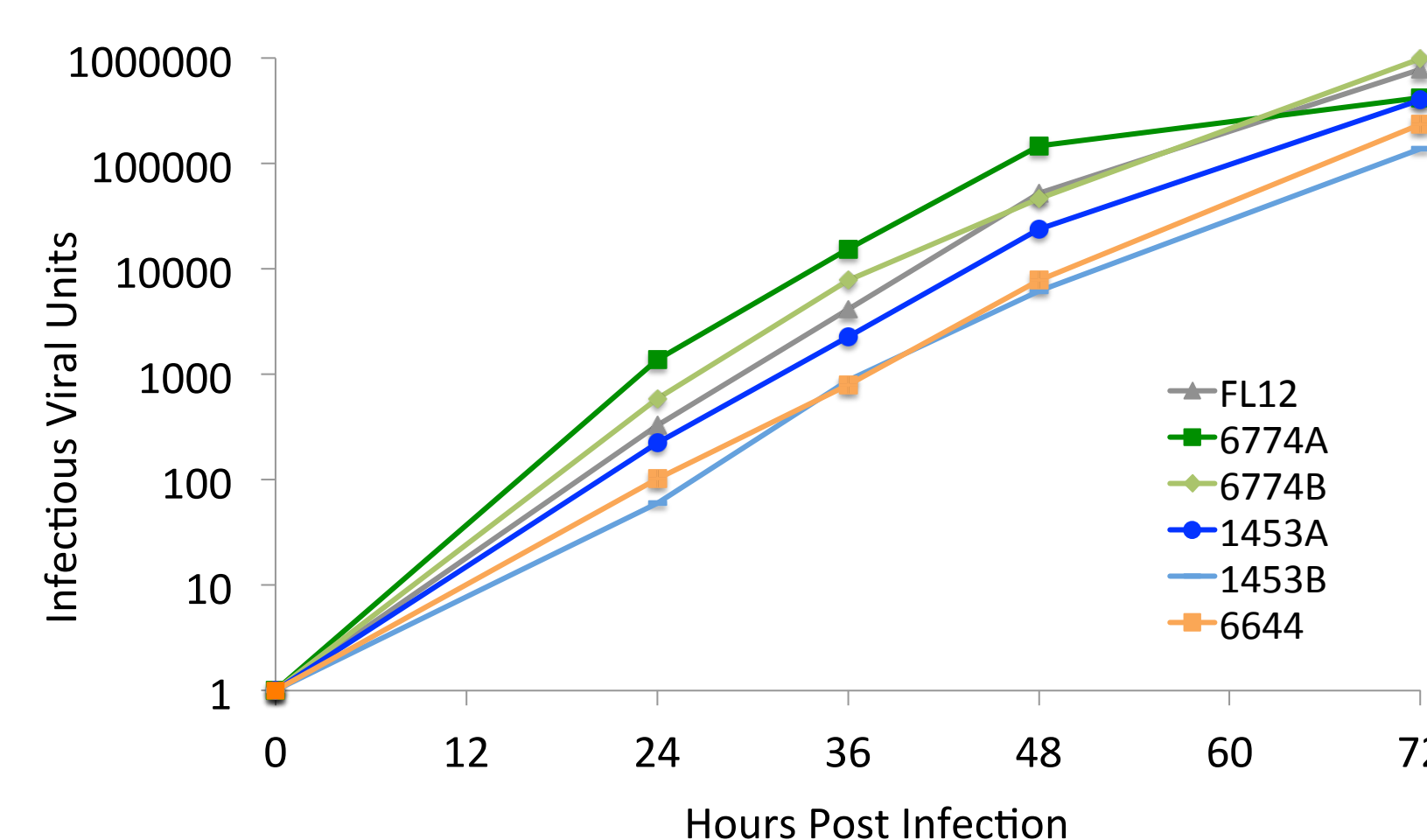


5. Replication Kinetics of PRRSV Variants



- Replication rates of rebound variants and FL12 were tested in MARC-145 cells
- Cells were infected with an equal number of infectious units of each virus
- Supernatants were harvested at sequential time points through 72 hpi and titered for infectious virus

- The 6774A rebound variant replicated faster than FL12
- The 1453B and 6644 rebound variants replicated more slowly than FL12
- PRRSV variants differ in replication kinetics, but differences are not associated with an immune escape phenotype



6. Summary and Conclusions

- Immune escape in PRRSV variants is associated with a decrease in infectivity
- PRRSV variants differed in replication kinetics, but differences were not associated with an immune escape phenotype
- Genetic changes in the PRRSV envelope that confer immune escape are associated with reduced replicative fitness

7. Acknowledgements

Many thanks to the University Honors Program for the opportunities in scholarship and research.

We thank the PRRS Host Genetics Consortium for providing pig serum samples and the NVSL97-7895 inoculum virus.

We thank Dr. Fernando A. Osorio, Dr. Asit K. Pattnaik, and Dr. Byung Kwon for the pFL12 plasmid, and for advice in construction of chimeric infectious clones and virus production.

Funding Sources: USDA, NIFA, HEP, National Needs Graduate Fellowship 2011-38420-20050 and The National Pork Board Projects #12-173, #14-222