Field and Experimental Assessment of PRRSV Evolution

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Summary and Implications

The genetic and antigenic stability of porcine reproductive and respiratory syndrome (PRRS) virus during infection in pigs was assessed using field and experimental data. Among field isolates, we found that PRRS virus varied both genetically and antigenically. Even within the same farm, various phenotypic strains of PRRS virus were present simultaneously. Experimentally, we demonstrated that PRRS virus "quasi-species" appeared over time as the virus replicated in animals. Although the degree of genotypic changes in the gene coding for the major envelope protein (i.e., open reading frame 5) was less than expected based on field observations; some mutants appear to have been changed significantly. Overall, our observations suggest that persistence of PRRS virus in pigs may be attributed to viral mutation, although the actual role of viral mutation in persistent PRRS virus infection remains to be determined. The presence of various phenotypic strains within the same farm or herd may account for the apparent ineffectiveness of PRRS control by monovalent vaccine.

Introduction

Unrecognized prior to 1991, porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant diseases of swine in the world today. A newly emerged arterivirus, PRRS virus causes reproductive losses in adult animals and respiratory disease in pigs of all ages (2,8). In acute outbreaks, economic losses from PRRS virus have been estimated to range from $236 to $502 per sow in farrow-to-finish and breeding stock operations (5). In response to the economic effects of PRRS, various management strategies and vaccination protocols have been tested for controlling PRRS. At present, the definitive solution to the prevention and control of PRRS has not been found.

Several characteristics of PRRS virus have been identified. The virus is highly infectious (13) and preferentially replicates in host macrophages (8). Infection results in humoral and cellular immune responses, but infectious virus can be recovered from pigs for several months after the initial exposure (9,14). Subclinically infected carrier animals are considered to be the key to the perpetuation of PRRS virus in endemically infected herds. At present, the exact mechanism by which PRRS virus evades the immune system is unknown. However, in other RNA viruses, persistent infections appear to be based on continuous mutations that select for viral "quasi-species" best adapted to continuous replication in certain cells (3). That is, the virus changes over the course of infection within an individual. The observation that PRRS virus field isolates vary genetically and antigenically suggest that a similar mechanism might be involved (1,3,4,7,10–12). To evaluate the dynamics of PRRS virus in individual animals and in populations, field-based and experimental studies were conducted.

Materials and Methods

Assessment of genetic and antigenic diversity among field isolates of PRRS virus. Field isolates of PRRS virus were obtained from clinical submissions to the Iowa State University Veterinary Diagnostic Laboratory between 1996 and 2000. Isolates were selected for genetic and/or antigenic analysis based on two criteria: the origin of isolates (i.e., viruses from the same herd or farm) or the restriction fragment length polymorphism (RFLP) pattern. Based on the first criterion, six isolates were chosen, which were recovered in a 2-month period from acutely affected pigs at different sites on the same farm. To avoid the introduction of PRRS virus strains into the herd, the management only accepted replacement animals from a single PRRS virus-negative source. The six isolates were first assayed for genetic variability using RFLP analysis (6) and then for antigenic differences using a panel of PRRS virus-specific monoclonal antibodies (10,11). Based on the second criterion, a total of 21 field isolates with RFLP cutting pattern 1-4-2 were selected for comparison. All isolates were recovered from swine herds in Iowa. Open reading frame (ORF) 5 of these isolates was sequenced and compared. ORF5 encodes for the major envelope glycoprotein and is known to be the most variable among isolates (1,4).

Assessment of genetic and antigenic changes of PRRS virus in pigs over time. A study consisting of a series of pig passages (n = 7) of PRRS virus was conducted to assess the degree and rate of virus mutation in pigs over time. Each passage consisted of four pigs, with each animal individually housed in a HEPA-filtered isolation unit. In passage 1, three pigs were inoculated with a plaque cloned PRRS virus derived from ATCC VR-2332, the prototypic North American isolate, and one pig served as a mock-infected control. The pigs were kept in isolation for 60 days post inoculation. During this time, serum samples were collected periodically from all pigs for virological and serological monitoring. After 60 days, each pig in passage 2 was inoculated with tissue homogenate filtrates from the corresponding pig in passage 1. This process was repeated

for each subsequent passage. At passages 1, 2 and 7, 15 plaque clones were collected from each inoculated pig and compared with respect to the ORF5 nucleotide sequence and their susceptibility to neutralising activity of antiserum collected at the end of the first passage. Plaque clones (n = 15) from the original inoculum were used as a baseline for comparison.

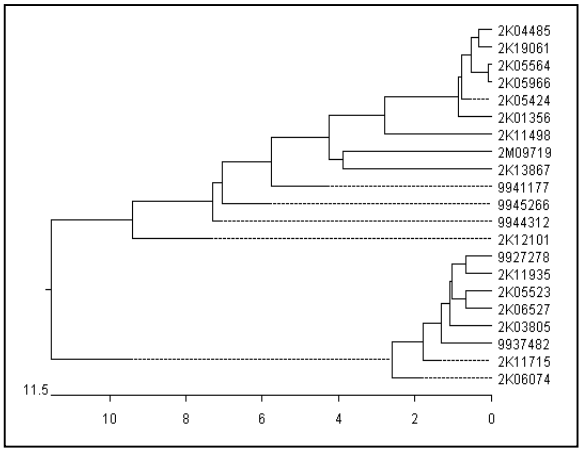

Figure 1. Variability of ORF5 nucleotide sequence among PRRS virus field isolates which had the same RFLP cutting pattern.

Results and Discussion

PRRS virus field isolates were found to vary genetically and antigenically. The fingerprinting analysis of six isolates selected from the same farm demonstrated that all isolates had the RFLP pattern designated 1-4-2. However, monoclonal antibody analysis found that the six isolates fell into four antigenic groups, indicating extensive phenotypic variability among these isolates, although they came from the same farm.

A comparison of the 21 isolates with the same RFLP pattern found that the percentage of sequence identity of ORF5 among the isolates ranged from 84 to 98%. Amino acid substitutions occurred most frequently in N terminal ends. A computer-aided phylogenetic analysis revealed two genotypic clusters (Fig 1), suggesting that the isolates were from two distinct origins. However, genotypic variability among the isolates and between two clusters did not correlate with geographical proximity or chronological order of isolation. Collectively, these observations suggested that the presence of various phenotypic endogenous strains within the same farm or herd could account for the apparent ineffectiveness of PRRS control by monovalent vaccine.

Experimentally, PRRS virus "quasi-species" appeared over time as the virus replicated in animals. All experimentally inoculated pigs harboured infectious virus at 60 days post inoculation, i.e., transmission to the subsequent passage was successful. Monitoring of viremia and antibody response at each passage did not reveal significant differences in the level of virus replication among pigs or between passages. Genetic changes in ORF5 were detected over time (Fig 2). Although the degree of changes were relatively small as compared with those found among field isolates**,** some isolates appear to have been changed sufficiently to escape serum neutralizing antibodies conferred by the initial infection.

Collectively, our observations suggested that viral mutation may be a mechanism of PRRS virus persistence. However, questions regarding the rate of mutation, type of mutation, and genetic "hot spots" of mutation remain to be addressed. Perhaps the most important question also remains, e.g., what is the actual role of viral mutation in PRRS virus persistence?

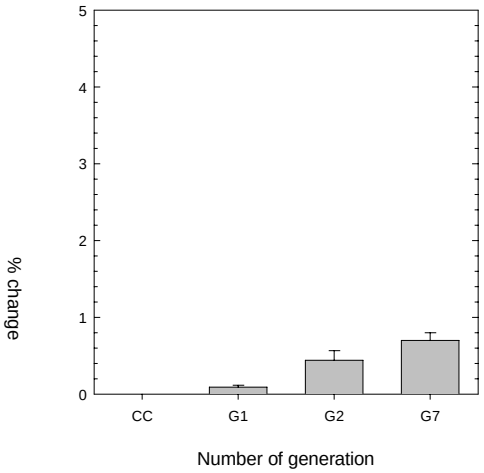

Figure 2. Rate of nucleotide change in ORF5 (603 nucleotide long) of PRRS virus in pig over time. Each vertical bar is the mean of 45 clones at each generation. Error bars are SEM.

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