Genetic and Antigenic Stability of PRRS Virus in Pigs

Field and experimental prospectives

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1. INTRODUCTION

Unrecognised 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 (Collins *et al* 1992, Wensvoort *et al* 1991). 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 (Polson *et al* 1992). 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 (Yoon *et al* 2000) and preferentially replicates in host macrophages (Wensvoort *et al* 1991). Infection results in humoral and cellular immune responses, but infectious virus can be recovered from pigs for several months following the initial exposure (Wills *et al* 1997, Zimmerman et al 1992). 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

The Nidoviruses (Coronaviruses and Arteriviruses).
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for viral "quasi-species" best adapted to continuous replication in certain cells (Domingo and Holland 1997). That is, diversity occurs 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 (Andreyev *et al* 1997, Murtaugh *et al* 1998, Wensvoort *et al* 1992, Yang *et al* 1999; Yang *et al* 2000, Yoon *et al* 1997). To evaluate the dynamics of PRRS virus in individual animals and in populations, field-based and experimental studies were conducted.

2. GENOTYPIC AND PHENOTYPIC 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.

Six isolates were chosen based on the first criterion. These isolates were recovered in a two-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 6 isolates were first assayed for genetic variability using a fingerprinting technique described by Wesley *et al* (1998) and then for antigenic differences using a panel of PRRS virus-specific monoclonal antibodies. Individual monoclonal antibodies represented distinct epitopes on the major structural (N, M, E) and 43kD proteins of PRRS virus (Yang *et al* 1999, Yang *et al* 2000). The fingerprinting analysis demonstrated that all

Table 1. Diversity of PRRS virus isolated from one herd as determined by monoclonal antibody analysis

PRRSV isolate	Month	Monoclonal antibody panels											
		15kD					19kD		25kD			43kD	
		A	B	C	D	E	A	B	A	B	C	A	B
VDL 98-16736	5	+	+	+	+	+	+	-	+	+	+	-	-
VDL 98-17455	5	+	+	+	+	+	+	+	+	+	+	+	-
VDL 98-19622	6	+	+	+	+	-	+	+	+	+	+	+	+
VDL 98-22148	7	+	+	+	+	+	+	+	+	+	+	+	-
VDL 98-24002	7	+	+	+	+	+	+	+	+	+	+	+	+
VDL 98-24104	7	+	+	+	+	+	+	+	+	+	+	+	+

isolates had the RFLP pattern designated 1-4-2. In contrast, monoclonal antibody analysis (Table 1) categorised the 6 isolates into 4 antigenic groups, indicating extensive phenotypic variability among these isolates.

In a second study, a total of 21 field isolates with RFLP cutting pattern 1-4-2 were selected based on the second criterion. All isolates were recovered from swine herds in the state of Iowa. Open reading frame (ORF) 5 of these isolates was sequenced and compared. ORF5 encodes for the major envelope glycoprotein (25kD) and is known to be the most variable among isolates (Andreyev *et al* 1997; Murtaugh *et al* 1998). The percent sequence homology among the isolates ranged from 84% to 98%. Amino acid substitutions occur more 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 2 clusters did not correlate with geographical proximity or chronological order of isolation.

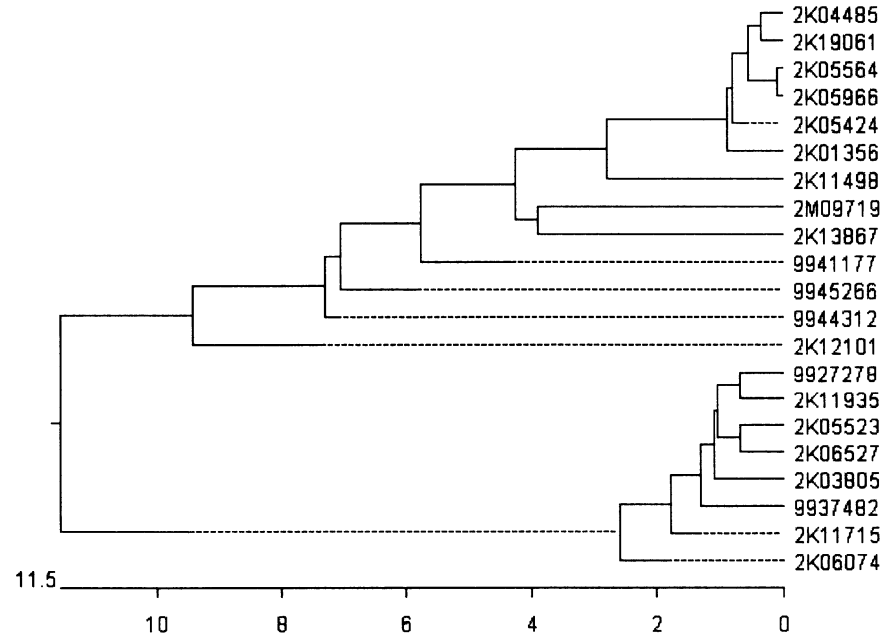

Figure 1. Variability of ORF5 nucleotide sequence among PRRS virus field isolates which had the same RFLP cutting pattern

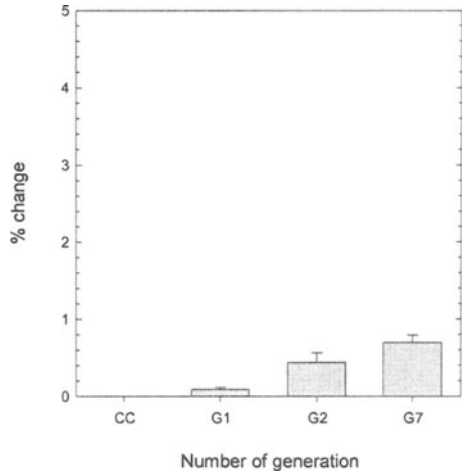

Figure 2. Rate of nucleotide change in ORF5 (603 nucleotide long) of PRRS virus in pig over time. Each vertical bar is the mean of 45 clones at each generation. Error bars are SEM.

3. GENETIC AND ANTIGENIC CHANGES OF PRRS VIRUS IN PIGS OVER TIME

Field isolates of PRRS virus show a remarkable degree of genetic and antigenic variability, but the degree and rate of virus mutation in pigs over time has not been characterized. To address this question, a study consisting of a series of pig passages (n = 7) of PRRS virus was conducted. Each passage consisted of 4 pigs, with each animal individually housed in a HEPA-filtered isolation unit. In passage 1, 3 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 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. All 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 virus replication during passages. Plaque clones (n = 15) from the original inoculum and clones (n = 135) from pigs at passages 1, 2, and 7 were

collected and compared with respect to ORF5 nucleotide sequence and their susceptibility to neutralising activity of antiserum collected at the end of the first passage. Genetic changes in ORF5 were detected over time (Fig 2), although the degree of changes were relatively small as compared to that found among field isolates. In addition, preliminary analysis of one-way cross neutralisation data suggested that escape mutants appeared during animal passage.

4. CONCLUSION

A descriptive study of field isolates clearly demonstrated that PRRS viruses vary genetically and antigenically. Subsequent experiments demonstrated that PRRS virus "quasi-species" appear over time as the virus replicates in animals. Although the degree of genotypic changes in ORF5 was less than expected based on field observations; some mutants appear to have been changed sufficiently to escape serum neutralizing antibodies conferred by the initial infection. Collectively, our observations indicate that viral mutation may be a mechanism of PRRS virus persistence. However, the rate, type, and "hot spots" of mutation remain to be addressed. Other questions remain to be addressed, as well. In particular, what is the actual role of viral mutation in PRRS virus persistence? And could the presence of various phenotypic endogenous strains within the same farm or herd account for the apparent ineffectiveness of PRRS control by monovalent vaccine?

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