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# Using GIS and sequencing to assess regional epidemiology of PRRSV

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a disease with an important economic impact worldwide. In the USA alone, the losses are calculated at $600 million yearly (NPB, 2003). These losses are due to a reduced reproductive performance and to higher rates of post-weaning mortality and poorer productive behavior in the growing-finishing stage (Joo et al., 1993; Polson et al., 1994; Zimmmerman et al., 1997). However, productive outcomes and clinical manifestations of the disease vary greatly among farms. Such variation is due, in part, to modifications in the genetic material of the PRRSV (Murtaugh et al., 2002). Modifications can be originated by mutations in the viral genoma or by recombination (mixture) of genetic material between virus (Murtaugh et al., 2001). This situation has important implications when producers try to control the disease in their herds. Infecting pigs with a specific strain of PRRSV or vaccinated them with available vaccines does not ensure complete protection against field challenges over time. Knowing which strains exist in their farms, people have an important piece of information when taking decisions to manage this disease. A way to measure the similarity between isolates is sequencing the ORF 5 region of the PRRS viral genoma (Torremorell, 2001). Generally, isolates with 98% or higher homology are considered of the same strain (Roberts, 2001). However, there are cases where the similarity in the PRRSV genomic composition between a farm and its source is lower than 95%, raising the possibility that the virus was introduced to the farm form other outside sources by unknown means.

The transmission of the PPRSV into a herd may follow several routes, including pigs (Dee et al., 1997), semen (Christopher-Hennings et al., 1995), contaminated fomites (Otake et al., 2002), and infected vectors like mosquitoes (Otake et al., 2002) and flies (Otake et al., 2003). Some other pathways, like visitors (Amass et al., 2000) and birds (Zimmerman et al., 1997; Trincado et al., 2003) have not been conclusively demonstrated. A potential mode of transmission of PRRSV among farms, aerial spread, is a controversial question. Mortensen et al. (2002) conducted a study in a cohort of 1071 sow herds, reporting that some factors, like exposure from virus-infected neighboring herds and increased herd size, significantly increased the risk of PRRSV infection. In this study, the authors suggest that virus spread via aerosols is a frequent means of PPRSV transmission among herds. Other authors also suggest the probability of aerial transmission among farms, mainly in regions with high density of swine population (Le Poitier et al., 1997; Lager et al., 2002). However, Golberg et al. (2000) did not find a correlation between geographic proximity and genomic similarity in 55 PRRSV isolates submitted by veterinarians from Illinois and eastern Iowa. This lack of correlation does not support the hypothesis of aerial spread (distance-related phenomena) of PRRSV. The purpose of our study was to investigate the patterns of PRRSV distribution among the sites of a single company using spatial analysis methods and PRRSV sequencing techniques. We describe two processes: the relationship between geographic distance and percentage of homology in the PPRSV genomic sequence among farms, and the relationship between the regional pig density for each site and the genomic similarity of the site with its source farm.

## Materials and methods

PRRSV's ORF 5 genomic sequences (n=62), processed in the Animal Disease Research and Diagnostic Laboratory of South Dakota State University, were obtained from a pork production company. The isolates were submitted for diagnostic testing from 2 January 2002 to 22 May 2003. The genomic sequences were aligned with the Clustal X computer program (Thompson et al., 1997) and the resultant PHYLIP report was displayed as a phylogenetic tree diagram (Figure 1) by the TreeViewWin32(c) program (Page, 2001).

The ClustalX alignment file was saved as MSF format and loaded into the DNAStar® program to generate a matrix of genomic distances between isolates.

Geographic coordinates of farms in decimal degrees for longitude and latitude were obtained from the company's database. Locations were displayed on a map using the software ArcView 8.2 (ESRI, Redlands, California). The coordinates were given at the fifth decimal place to allow a tolerance not higher than 1.1 meter when plotting the locations on the map. NDA 1983 State Plane Coordinate Systems were applied to generate the maps (Figure 2).

Figure 1. Phylogenetic tree diagram generated by TreeViewWin32(c) for the 62 genomic sequences

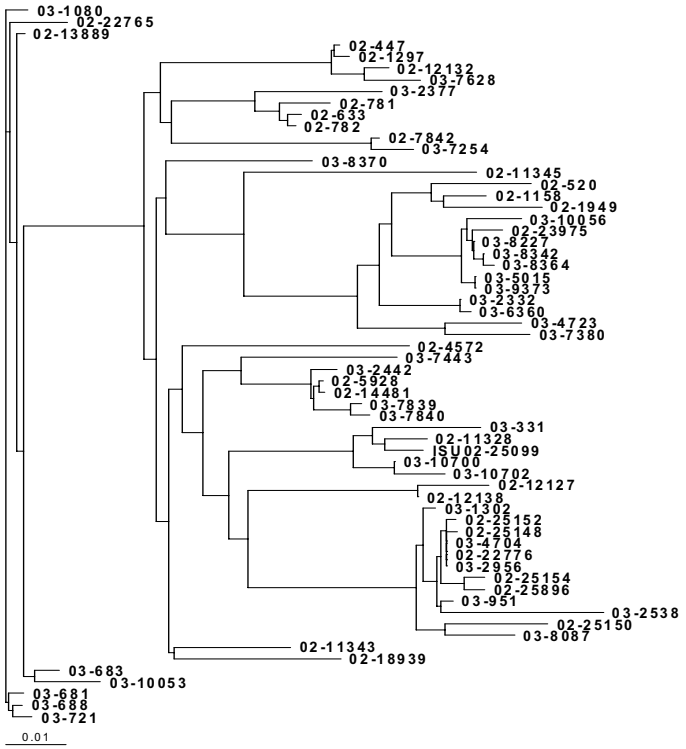
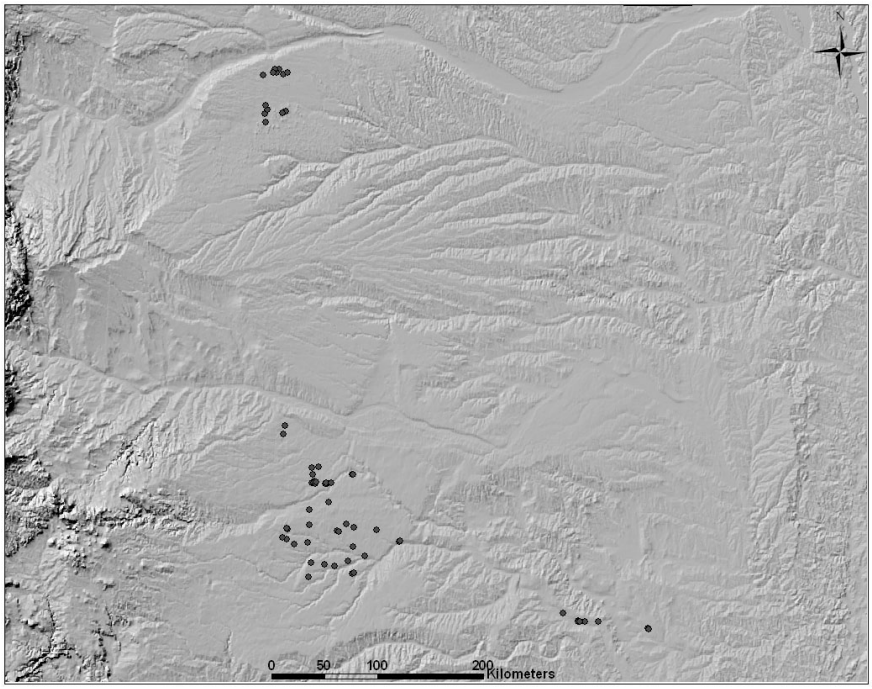



Figure 2. Map displaying the position of the 62 sites in study




Distances between sites were calculated in an Excel® spreadsheet using the great circle formula (Geoscience Australia, 2003).

The matrix of genomic distances generated by the DNAStar® program was compared with the geographic distances generated in the Excel® spreadsheet to analyze the relationship between the geographic distance and the

genomic distance among the 62 farms. The 1891 pairs of observations were analyzed with the STATISTICA® package to obtain the coefficient of correlation and generate the graphic analysis.

Recent studies applying swine population density in their analysis have used buffers of 6km radius (Tamba et al., 2002), and of 5km radius (Mortensen et al., 2002). In this case, the regional population density by site was calculated applying a radius of 5.6419 km from each site (Figure 3), to measure both the number of animals and the number of herds per 100km$^2$. The relationship between the regional swine population for each of the 62 sites and the genomic distance between the site and its source farm was analyzed using the STATISTICA® package to obtain the coefficient of correlation and generate the graphic analysis.

In our analysis of density we only used the number of animals per 100km$^2$ because we found that for this system the density for animals and for herds was strongly correlated (r=0.8767).

## Results

### Relationship between geographic distance and percentage of homology in the PPRSV genomic sequence among farms.

A total of 1891 pairs of observations were analyzed to determine the correlation between geographic distance and genomic similarity among 62 farms (r=-0.2452, *P*<.01) For this company, geographic distance appears to be a factor associated with genomic divergence among farms.

### Relationship between the regional pig density for each site and the genomic similarity of the site with its source farm.

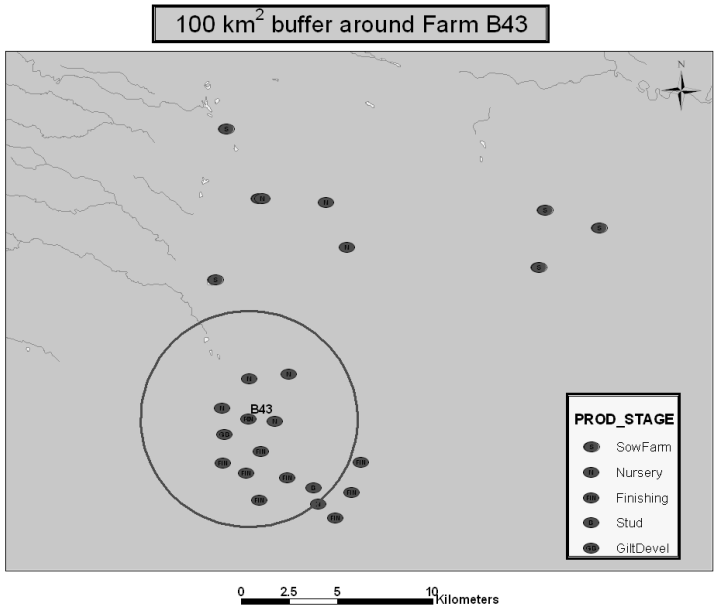
Data on the number of animals within a radius of 5.6419km was obtained for each of the 62 farms in this study. From January 2002 to May 2003, the farms of this company located in areas of higher swine population density show a lower genomic similarity with their source farm when they are compared with other farms of the same company located in areas of lower swine population density (r=-0.3547, *P*<.01)

As an additional finding to the last two analyses we found that the genomic similarity of the site with its source farm differs depending on its position downstream the flow of production (*P*<.05). The sow farms had a 95.4% average of genomic similarity with their sources, while the growing-finishing sites had a 92.9% average of genomic similarity with their sources.

## Discussion

Some factors frequently associated with presumed lateral transmission of disease between farms such as geographic distance and regional animal density may influence the genomic variability among the farms of this company. More studies are needed to validate the significance of these results. At the moment we are building a bigger database with more information supplied by the same source. An increasing number of observations will confer a higher validity to the results of future analyses. Additionally, we need to investigate the correlations among the factors analyzed in an independent way in this paper (distance, density, flow) and the viral variances of the

Figure 3. Map of Section B from the pork producer company in study—the map shows the buffer of 5.6419km radius (or 100km$^2$) from Farm B43



farms to get a broader understanding of the patterns of distribution of PRRSV.

The substantial increase in genomic diversity in the system suggests that the presence of neighboring farms not belonging to the company in this study may be a source of new viral introductions. It is important for future studies to incorporate all the possible information related with neighboring sites to bring more reliability to the results. As other producers and practitioners show interest in share their data, spatial analyses on regional PRRSV transmission will gain in accuracy.

Is very important to understand the behavior of the variables affecting the movement of PRRSV among herds to develop effective strategies to control the disease. If the data available suggest evidence of regional spread of the disease, control strategies should be integrated on processes coordinated in time by common areas instead of several isolated efforts at different times.



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