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Immunological features of porcine reproductive and respiratory syndrome virus (PRRSV) against homologous and heterologous challenge in a population of breeding age female swine

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is one of the major disease problems in the swine industry today (Loula, 1991; Dee et al., 1997). PRRSV, the causative agent, is a small, enveloped, positive-strand RNA virus classified in the order *Nidovirales*, family *Arteriviridae*. Other family members include equine arteritis virus, lactate dehydrogenase-elevating virus, and simian haemorragic fever virus (Plagemann and Moennig, 1992). These viruses replicate primarily in macrophages and are known to establish asymptomatic persistent infections in their hosts. PRRSV persistence involves a continuous low level of viral replication but is not a true steady-state persistent infection (Domingo et al., 1998; Allende et al., 2000). Two distinct genotypes of PRRSV evolved independently in Europe and North America (Meng et al., 1995; Murtaugh et al., 1995; Nelson et al., 1999). Studies of PRRSV variation within North America have established that the virus is antigenically (Yoon et al., 1997) and genetically (Meng et al., 1995; Kapur et al., 1996) highly diverse. The ORF 7 gene encoding the nucleocapsid (N) protein is conserved within major genotypes, compared to the major envelope gene (GP5) product of ORF 5 which exhibits substantial genetic diversity. Due to these differences, field isolates are referred to as strains, even though there is no consensus on what constitutes a strain (Murtaugh and Faaberg, 2001; Mengeling et al., 2003). Even though PRRSV strains isolated from around the world cause similar diseases in pigs, increasing data indicates that PRRSV strains differ in virulence in infected animals and are antigenically and genetically heterogeneous—witness, for example, the outbreaks of atypical PRRSV characterized by abortions and high mortality in pregnant sows experienced in the U.S. and even in farms were the breeding herd had been recently vaccinated (Mengeling et al., 1998).

To date most of these differences among the North American strains have been determined on the bases of sequencing (Meng et al., 1995), restriction fragment polymorphism (RFLP) analysis (Wesley et al., 1998), and serological cross reactivity using monoclonal antibodies and in vitro procedures (Yoon et al., 1995). PRRSV sequencing is useful because it permits the reconstruction of patterns of viral change in the field. It is often possible to determine if virus strains recovered from farms originated from pre-existing isolates or represent new introductions from outside sources. Similarly, analysis of viral sequences may indicate if a strain of virus represents a form of vaccine virus that was re-isolated from a vaccinated herd. Most of the information derived from these studies has been applied to epidemiological investigations, nevertheless homologous and heterologous immunity knowledge has not been improved though these techniques. At present the relationship between nucleotide or amino acid sequence variation and RLFP patterns is not known. Therefore, it is not possible to associate specific genetic characteristics of a PRRSV strain with biological or immunological properties or characteristics (Murtaugh and Faaberg, 2001). PRRSV classification has not evolved further than defining North America, European, vaccine-like, or field virus (Andreyev et al., 1997). Defining strains by degree of homology is highly subjective. There are several reports of imprecise guidelines to the interpretation of ORF 5 sequences. A homology of 99–100% is evidence that two sequences are identical and (94% homology indicates that genomic differences are great. This of course leaves an undetermined range of 94–99% (Collins, 1998). A less confident but more concise guideline for interpretation is that similar sequences are (97% homologous and dissimilar sequences are (97% homologous (Roberts, 2001). Unfortunately sequencing does not provide information related to virulence, antigenic similarity, or the degree of immunity conferred by infection of swine with one strain of PRRSV against another strain of PRRSV. Nevertheless, it is very common that in the field clinicians use the sequencing or RFLP information to establish homology levels between different strains isolated either in the same farm or the same company and, therefore, to decide if combined flow production is adequate to implement control measurements against PRRSV—that is, the controlled exposure of gilts (Batista et al., 2002) or sows (Desrosiers, 2000) to the homologous PRRSV strain—and to support their decision of vaccination against PRRSV.

Assessment of PRRSV of homologous and heterologous challenge

Objectives

To document the dynamics of both humoral and cellular immune responses, in both naive and previously PRRSV challenged gilts, when re-challenged with different strains of PRRSV.

Material and methods

- Sixty 4-month-old female swine originating from a PRRSV-naive source were inoculated on day 0 with the field isolate MN-30100 at a concentration of $10^{2.4}$ $TCID_{50}$ (total dose).
- Animals were bled on days 0, 3, 7, 21, 30, 60, 70, 90, 100, 110, 120.
- On day 120, animals were allocated into 6 different groups (10 animals per group) as follows:
    - Group 1: MN30100 non-challenged group
    - Group 2: MN30100 re-challenged group
    - Group 3: 10221-4, 3.4% sequence heterology (heterology here, and below, as defined by sequence of the ORF-5 PCR product)
    - Group 4: 25616-32, 5.4% sequence heterology
    - Group 5: VR2332, 16.5% sequence heterology
    - Group 6: 1-8-4, 11.3% sequence heterology
- Animals were bled on day 0, 3, 7 and 14 post-inoculation of the second (homologous or heterologous) challenge.
- Also on day 0 15 (PRRSV-naive control animals were allocated into 5 groups and challenged with:
    - Group 1: MN30100 re-challenged group
    - Group 2: 10221-4, 3.4% sequence heterology (heterology here, and below, as defined by sequence of the ORF-5 PCR product)
    - Group 3: 25616-32, 5.4% sequence heterology
    - Group 4: VR2332, 16.5% sequence heterology
    - Group 5: 1-8-4, 11.3% sequence heterology
- Serum was assayed for VI, PCR, and PRRSV ELISA antibodies and PBMC were analyzed for the presence of IFN-(-producing cells by ELISPOT and flow cytometry.

Results

- Figure 1 shows the humoral response measured by ELISA to PRRSV following homologous and heterologous challenge of the previously inoculated PRRSV experimental animals. All groups except group 1 presented and anamnestic antibody response to both the homologous and heterologous challenge, the lowest response was present in group 2 and the highest in group 4.
- Figures 2 and 3 show the percentage of animals—in the naive challenge group and the previously infected-heterologous groups, respectively—that had fever from days 0–15 of the experiment. The naive groups had fever in all groups and in a higher proportion of animals compared to the previously infected heterologous group.
- Figures 4 and 5 show the viremia detected in the naive challenge and previously infected heterologous groups. The naive groups presented viremia on all days tested in practically 100% of the animals. In contrast, the previously infected heterologous group

Figure 1. Mean ELISA S:P ratios of the homologous and heterologous challenge groups on days 0–14 of the experiment

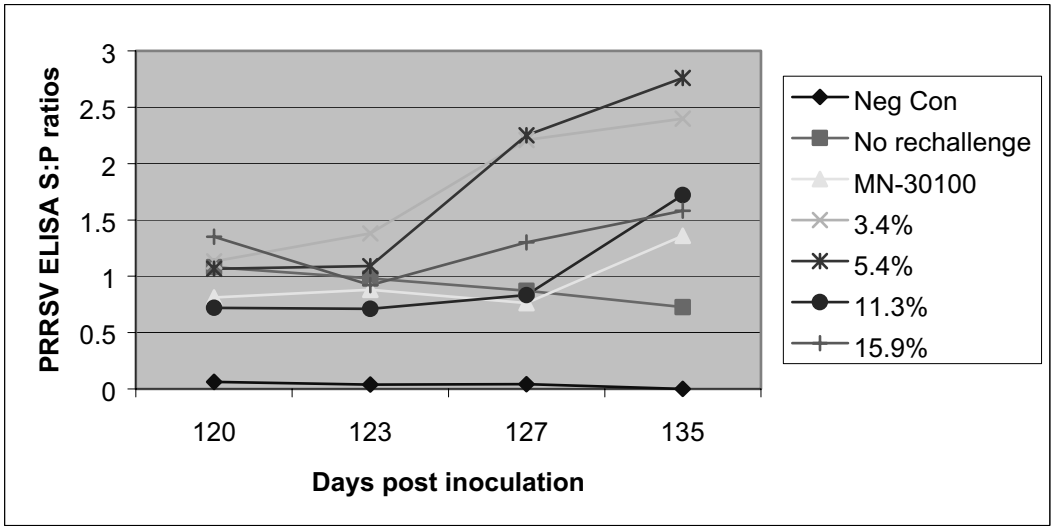


Figure 2. Percentage of animals in the previously infected homologous and heterologous challenge groups that presented fever from days 0–14 of the experiment

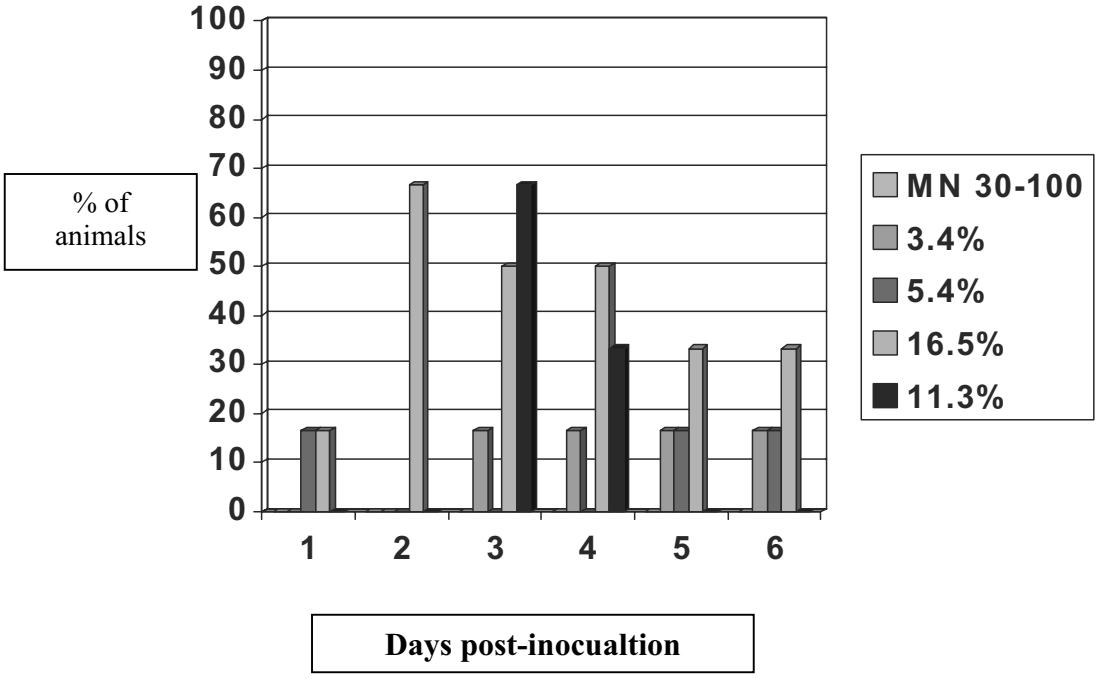
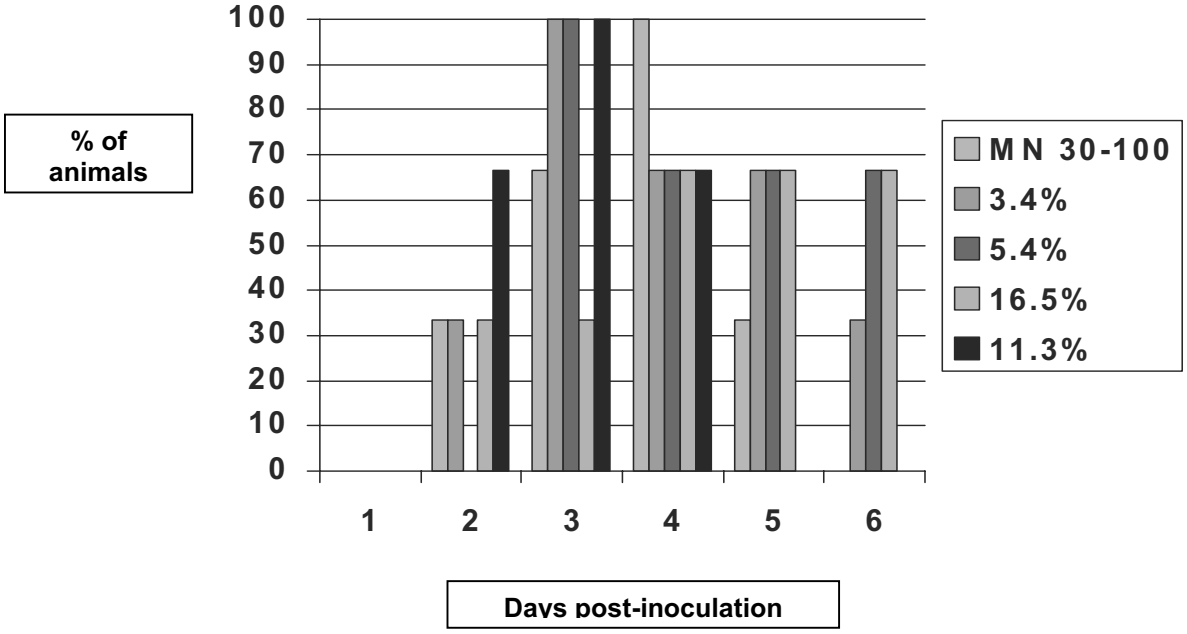
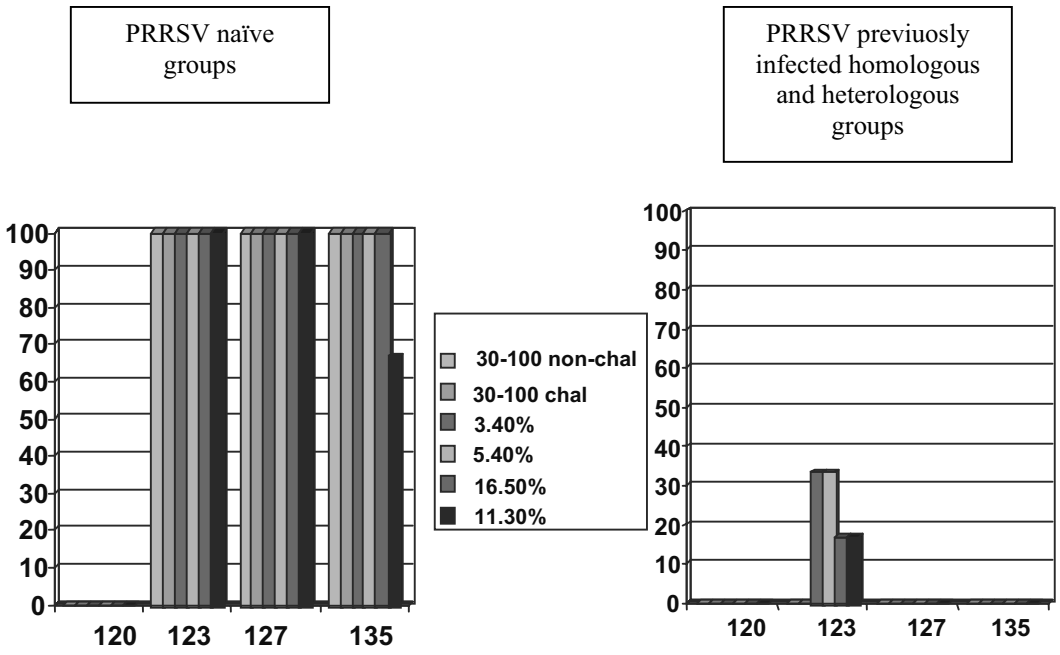


Figure 3. Percentage of animals in the naive groups that presented fever post-inoculation from days 0–14 of the experiment



Figures 4 and 5. Percentage of viremic pigs in the naive challenge groups (left) and in the previously infected homologous and heterologous groups (right) on days 0–14 post-inoculation



presented a mild transient viremia in all the groups but only in day 3 of the experiment.

- Finally figure 6 shows the cellular response to the homologous and heterologous PRRSV challenge. In contrast to the humoral response the homologous group presented an elevated and rapid CMI response >10% of IFN-(-producing cells. Groups 3 and 5 presented a mild CMI response while groups 4 and 6 remained at basal levels.

Conclusions

- Viremia was detected in all the previously infected heterologous groups on day 3 pi, but not at days 7 and 14 pi. It should be noted that there are reports of detection of viremia on day 1 in previously infected homologous challenge (McCaw, 2003). In this experiment animals were not bled on day 1 therefore we cannot make any comparisons or conclusions on the presence of early, short-lived viremia.
- Clinical signs of fever (> 40˚C), anorexia, and depression were observed in groups 3 (3.4% heterology), 5 (16.5% heterology), and 6 (11.35% heterology) but not in other groups challenged with heterologous strains.
- Immunity protected against the homologous challenge in 100% of the animals and in at least 66% of the animals in all the heterologous challenge groups. This heterologous protection was robust, since these animals showed a transient viremia only on day 3
- In all the groups challenged with the heterologous strains, there was a rapid and high anamnesic antibody response on day 14 post-infection.
- There were no apparent differences in the humoral response in the different heterologous groups with an heterology ranging from 3.4–16.5% from the original challenge strain.
- The homologous challenge group presented a high and rapid CMI response while 2 groups in the heterologous challenge groups presented an intermediate response and 2 groups remained at the same levels they had before the heterologous challenge. The rapid CMI response in the homologous group could explain the lack of viremia in the homologous challenge.

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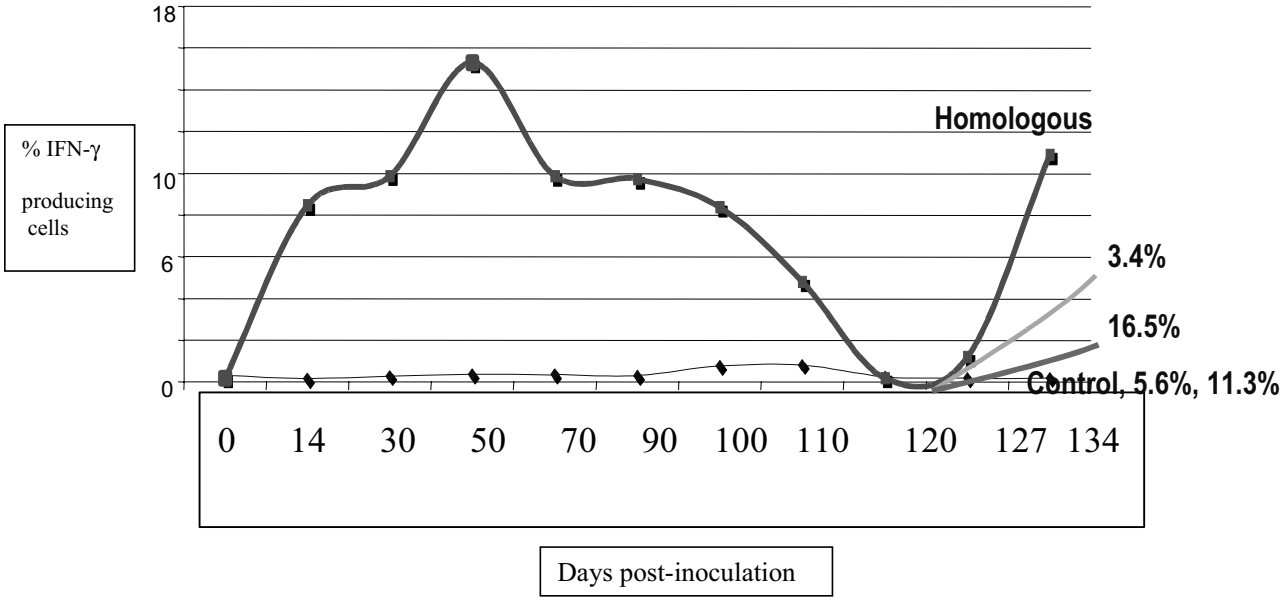
Figure 6. Total IFN-(production for the original,homologous, and heterologous challenge group from days 0 to 134 post-inoculation as reported using flow cytometry



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