

Title: Studies on PRRSV vaccination: interleukin-12 to enhance the immune response to killed vaccine and combination vaccination with MLV and killed vaccine - **NPB # 02-127**

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Date Received: June 2, 2003

Abstract: The efficacy of PRRSV vaccines remains controversial. The objective of this study was to investigate whether interleukin (IL) -12 could be used to enhance the immune response to a killed PRRSV vaccine (KV) and if a combination of a KV following administration of a modified live PRRSV vaccine (MLV) would result in a more rapid immune response compared to either vaccine alone. The study consisted of 5 groups of pigs inoculated with IL-12 only, KV only, KV + IL-12, MLV only, and KV following MLV, and a non-inoculated control group. Pigs were challenged with PRRSV at 42 days post vaccination (dpv) to evaluate protective immunity. The study demonstrated that IL-12 slightly enhanced the efficacy of KV as demonstrated by increased antibody levels measured by IDEXX ELISA and increased number of interferon-gamma producing cells. Interestingly, the combination of vaccines (KV following MLV) appeared to induce stronger antibody and lymphocyte proliferative responses compared to vaccination with either vaccine alone. This vaccination strategy resulted in higher serum neutralizing antibody levels than either vaccine alone on 42 dpv, although the difference was not statistically different. The magnitude of CD4⁺, CD8⁺, CD4⁺CD8⁺ lymphocyte proliferative responses were slightly higher in the combined vaccination group on 28 dpv and 7 days post infection (dpi) compared to all of the other groups. In addition, the combination vaccination reduced the magnitude and duration of viremia following PRRSV challenge.

The results of this study suggest that a protocol combining MLV and KV could increase the immune responses compared to either vaccine alone.

These research results were submitted in fulfillment of checkoff funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer reviewed

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Introduction and objective: Porcine reproductive and respiratory syndrome virus (PRRSV) continues to cause significant economic losses to the swine industry worldwide. Numerous protocols, including vaccination to increase herd immunity, have been developed to control and/or eliminate PRRSV. Unfortunately, the success of these programs has been variable and vaccine failure is commonly reported by producers and veterinarians in the field. Regardless of the type of vaccine, the efficacy of PRRSV vaccines remain controversial. Current commercial PRRSV vaccines include one killed virus vaccine (KV) and two modified live virus vaccines (MLV). Both types of vaccines fail to stimulate a rapid strong cellular immune response or induce significant and rapid serum neutralizing (SN) antibody titers following administration [1]. In addition, PRRSV vaccines are judged to be considerably less effective than vaccines used for controlling other swine diseases. Results generated from previous studies in our laboratory suggested that the use of KV in previously exposed pigs, either by natural infection or vaccination with MLV, was able to induce stronger cellular immune and SN antibody responses [2, 3].

The objective of this study was to determine if interleukin (IL) -12 added to PRRSV KV would enhance vaccine efficacy and the immune response to KV alone, and to investigate if KV following vaccination with MLV would result in a more rapid immune response compared to vaccination with MLV alone.

Materials and **Methods**:

- Vaccines and challenge virus

Ingelvac™ PRRS MLV vaccine (serial no. JA-746A-487; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was manufactured from the North American prototype PRRSV strain VR-2332 by serial attenuation in cell culture. PRRomiSe™ (serial no. 7252902, Intervet Inc., Millsboro, DE) was manufactured from PRRSV strain VR-2402. This virus strain was originally designated as ISU-P. Both vaccines were administered to pigs in accordance to instructions provided by the manufacturers. PRRSV strain ISU-P was used to challenge. The seed virus was provided by Intervet Inc.

- Experimental design

Determination of IL-12 dosage: Prior to conducting the principal experiment, the dosage requirement for the adjuvant effect of IL-12 was determined by vaccinating pigs with PRRSV KV containing different concentrations of IL-12. Sixteen two-to-three week-old crossbred pigs obtained from a herd free of PRRSV were randomly assigned to 4 treatment groups, 4 pigs per group. Group A served as a KV control (no IL-12 added). Pigs in this group were vaccinated intramuscularly on days 0 and 14 with PRRSV KV according to the manufacturer's directions. Groups B, C, and D were vaccinated intramuscularly on days 0 and 14 with 2 mL of KV containing 0.5, 3, and 5 µg of IL-12, respectively. Blood samples were collected on 0, 14, 28, and 42 day post vaccination (dpv). Phenotypic responses of lymphocytes and enumeration of interferon-gamma (IFN-γ) producing cells in peripheral blood mononuclear cells (PBMCs) were measured by flow cytometric assay. Antibody responses were measured by IDEXX ELISA and serum neutralization (SN) assay.

Principal experiment: Fifty two-to-three week-old crossbred pigs were obtained from a herd serologically free of PRRSV. Upon arrival, the pigs were randomly assigned to 5 treatment groups, 10 pigs per group. **Group 1** served as negative and IL-12 controls; half of the pigs in this group (n=5) was injected intramuscularly with 2 mL of sterile saline and the other half (n=5) was injected intramuscularly with 5 µg IL-12 (R&D

System, MN) diluted in 2 mL of sterile phosphate buffer saline containing 0.2% bovine serum albumin according to the manufacturer's direction. **Group 2** served as a KV control. Pigs were immunized with PRRSV KV according to the manufacturer's instructions on days 0 and 14. **Group 3** was a KV + IL-12 group. Pigs were immunized intramuscularly with 2 mL of KV formulated with 5 µg of IL-12 on days 0 and 14. **Group 4** served as a MLV control. Pigs were immunized with MLV according to the manufacturer's directions on day 0. **Group 5** was vaccinated with MLV on day 0 followed by 2 doses of KV on days 14 and 28 according to manufacturer's directions. All pigs were challenged intranasally with 2 mL of PRRSV strain ISU-P (10^4 TCID₅₀/mL, 1 mL per nostril) on 43 dpv (0 dpi, day post infection) to evaluate the level of protective immunity.

All pigs were evaluated for clinical disease daily following challenge. A clinical respiratory disease score was given to each pig according to method described by Halbur et al., 1995 [4]. Blood was collected from all pigs on 0, 28, and 42 dpv, and 7, 14, and 23 dpi. Sera were assayed for antibody responses by IDEXX ELISA and SN tests. Peripheral blood mononuclear cells were assayed for cell-mediated (CMI) responses by the lymphocyte proliferation assay using a green fluorescent dye (PKH67). Phenotypic responses of lymphocyte subsets were determined as well. Following challenge, sera were assayed for viral RNA using a quantitative real-time reverse transcriptase polymerase chain reaction (QRT-PCR). Oropharyngeal scraping samples collected at the time of blood collection, starting at 7 dpi and bronchoalveolar lavage (BAL) samples collected at necropsy were also assayed for virus and viral RNA by virus isolation (VI) and nested reverse transcriptase polymerase chain reaction (NRT-PCR), respectively.

Results:

Determination of IL-12 dosage: The antibody responses in pigs vaccinated with KV containing variable concentrations of IL-12, as determined by ELISA are summarized in Figure 1. The mean ELISA titers of pigs vaccinated with KV containing IL-12 were higher than that of pigs vaccinated with KV only; however, a linear correlation among the IL-12 dosages was not found. Groups receiving KV with 3, 5 and 0.5 µg of IL-12 displayed the highest through lowest magnitude of antibody responses, respectively. No PRRSV-specific SN antibody assay was detected in any of the pigs following vaccination. Following immunization with KV containing variable concentrations of IL-12, the mean percentages of CD8⁺ cells of all treatment groups increased. After reaching the highest level on 28 dpv, they decreased. In contrast, the mean percentage of CD4⁺ cells decreased overtime. No significant differences in percentages of CD8⁺ and CD4⁺ cells among treatment groups was detected. The mean percentage of IFN-γ producing cells by all groups is summarized in Figure 2. The number of IFN-γ producing cells increased, reaching the highest levels on 14 dpv and then declined. The mean percentage of IFN-γ producing cells in pigs vaccinated with KV plus IL-12 were higher than observed in pigs vaccinated with KV alone on 14 dpv. However, no statistically significant difference was observed. Within the groups receiving KV plus IL-12, the dosage of 0.5 and 3 µg had the highest and lowest numbers of IFN-γ producing cells, respectively.

The results of this preliminary study suggest that the addition of IL-12 to PRRSV KV could slightly enhance vaccine efficacy as demonstrated by increased antibody levels measured by ELISA and the number of IFN-γ producing cells. However, the magnitude of the augmented responses induced by IL-12 was not linearly related to the administered dosage.

Principal experiment: All groups inoculated with PRRSV displayed mild symptoms of respiratory disease consistent with PRRSV infection (Table 1). Clinical respiratory signs included labored and abdominal breathing. Control groups, which received saline or IL-12 only, had higher clinical scores. Vaccination with KV, alone or with IL-12 and/or MLV, resulted in a significant reduction of clinical disease following challenge. Pigs immunized with KV containing IL-12 displayed the mildest clinical disease. Antibody responses as measured by IDEXX ELISA are summarized in Figure 3. Prior to PRRSV challenge, only pigs vaccinated with MLV, either alone or in combination with KV, were seropositive (S/P ratio ≥ 0.4). Negative controls, pigs vaccinated with KV alone, or KV plus IL-12 remained seronegative. After challenge, only the non-vaccinated control and pigs vaccinated with KV or KV plus IL-12 exhibited increased antibody levels.

Results of SN antibody assays, using PRRSV strains ISU-P and VR-2332 as antigens are summarized in Figures 4 and 5. Prior to challenge, only pigs vaccinated with MLV or MLV followed by KV were positive for SN antibodies against both PRRSV strains. SN antibodies were first detected on 42 dpv. The level of antibodies against both PRRSV strains increased throughout the experiment. In contrast, the SN antibodies in the control groups, and pigs vaccinated with KV either with or without IL-12 remained undetectable after vaccination, resulting in a significant difference on 42 dpv. After challenge, the SN antibodies specific to both PRRSV strains increased in all treatment groups. Interestingly, control pigs and pigs previously inoculated with KV, either alone or with IL-12, exhibited increased SN antibody levels to PRRSV ISU-P more rapidly than to PRRSV VR-2332.

Cell-mediated immune responses were measured by lymphocyte proliferation assays coupled to a phenotypic evaluation of the lymphocyte subsets. Prior to vaccination, the magnitude of the CD8⁺, CD4⁺ and CD4⁺CD8⁺ lymphocyte proliferation responses were similar in all treatment groups. All vaccines resulted in increased magnitudes of CD8⁺, CD4⁺ and CD4⁺CD8⁺ lymphocyte proliferation responses over time. However, the magnitude of the proliferation responses was not different between the groups receiving the KV, which was slightly lower than the response of pigs receiving both vaccines on 28 dpv and 7 dpi. Following PRRSV challenge, the proliferation responses of CD4⁺ and CD4⁺CD8⁺ T lymphocytes increased in pigs that previously received any vaccine. The proliferation response of CD4⁺ T lymphocytes decreased after 7 dpi, whereas the response of CD4⁺CD8⁺ T lymphocytes declined after 14 dpi. In contrast, the proliferation response of CD8⁺ remained at or near the level prior to challenge.

Virus isolation and RT-PCR: Following challenge, administration of either MLV alone or in combination with KV reduced the magnitude and duration of viremia. None of the pigs in either MLV vaccinated group were positive using viral isolation techniques. Only 1 MLV vaccinated pig was PCR positive on 7 dpi. Pigs receiving either KV or KV + IL-12 also demonstrated reduction in the magnitude of viremia, but the difference was not statistical. In contrast to virus detection in serum, viral RNA was consistently detected by NRT-PCR in oropharyngeal scraping samples and BAL samples in all vaccine groups following challenge.

Discussion: The results obtained from this study demonstrated that administration of KV did not induce a strong antibody response, but CMI responses appear to occur as demonstrated by the increased T lymphocyte response. Adding IL-12 to the KV slightly enhanced ELISA antibody levels and the number of IFN- γ producing cells. The pigs vaccinated with KV plus IL-12 exhibited the mildest clinical disease. However no

reduction in the magnitude and duration of viremia was demonstrated suggesting incomplete protection against PRRSV challenge.

Interestingly, the administration of KV following MLV vaccination induced stronger humoral and CMI responses compared to vaccination with MLV or KV with or without IL-12. Serum neutralizing antibody and lymphocyte proliferative responses were also higher in the combined vaccination group as compared to other vaccine groups. In addition, the administration of both vaccines reduced the magnitude and duration of viremia following challenge with the homologous PRRSV strain. Clinical respiratory signs were significantly reduced in the vaccinated group as well.

The results of this study suggest the need for further investigation of protocols combining administration of MLV and KV vaccines in order to induce a higher magnitude of immune responses than those induced by either vaccine alone.

Lay interpretation: PRRSV vaccine efficacy remains controversial. The currently available PRRSV vaccines, either modified live (MLV) or killed virus (KV) vaccines, often appear to provide insufficient protective immunity. Therefore, this study was designed to investigate if interleukin (IL) – 12, a protein known to induce a cell-mediated immune (CMI) response, added to the KV would enhance vaccine efficacy. Combining MLV and KV (2 doses of KV following MLV) was also investigated to determine if this vaccination strategy would induce stronger and more rapid immune responses. The results of this study suggest that adding IL-12 failed to enhance the protection against PRRSV challenge. Addition of IL-12 slightly enhanced the efficacy of KV with regard to induction of humoral and CMI responses as demonstrated by increases of ELISA titer and the number of IFN- γ producing cells. It is noteworthy that administration of MLV followed by 2 doses of KV, 2 weeks apart, induced stronger humoral and CMI responses compared to vaccination with either vaccine alone. Pigs in the group receiving both vaccines displayed higher levels of serum neutralizing antibodies than pigs receiving either MLV or KV alone. However, no statistical difference was observed. The magnitude of T-lymphocyte proliferative responses was slightly higher in the group receiving both vaccines on 28 dpv and 7 dpi compared to the other vaccine groups. In addition, the magnitude and duration of viremia was observed following the administration of both vaccines was reduced.

The results of this study suggest that combining MLV and KV vaccination protocols may induce greater immune responses, resulting in reduced clinical disease and viremia compared to administration of either vaccine alone.

References:

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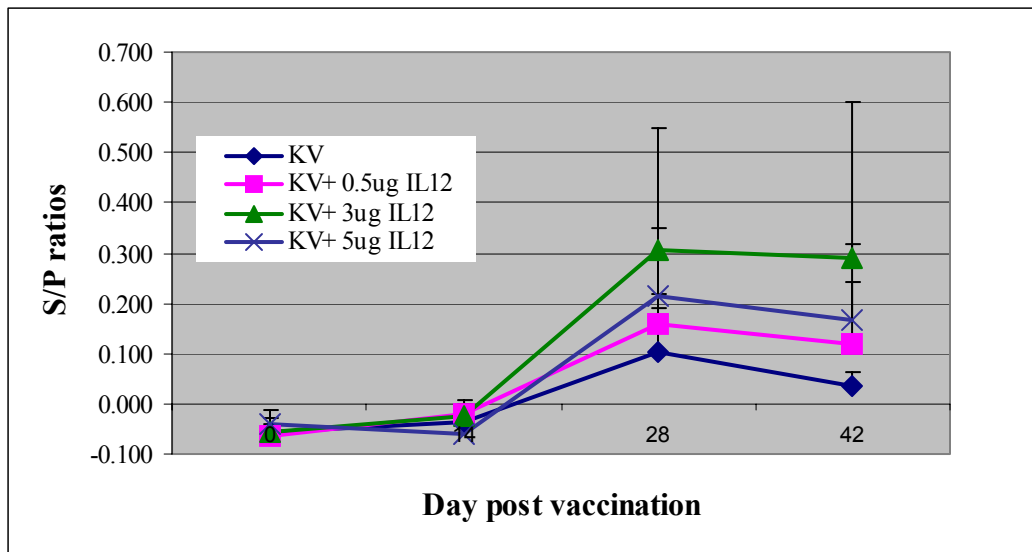


Figure 1. Antibody responses determined by ELISA in pigs vaccinated with a killed PRRSV vaccine (KV) containing variable concentrations of interleukin-12 (IL-12). Responses were expressed as mean of serum-to-positive (S/P) ratio + one standard deviation. An S/P ratio ≥ 0.4 is considered positive to PRRSV.

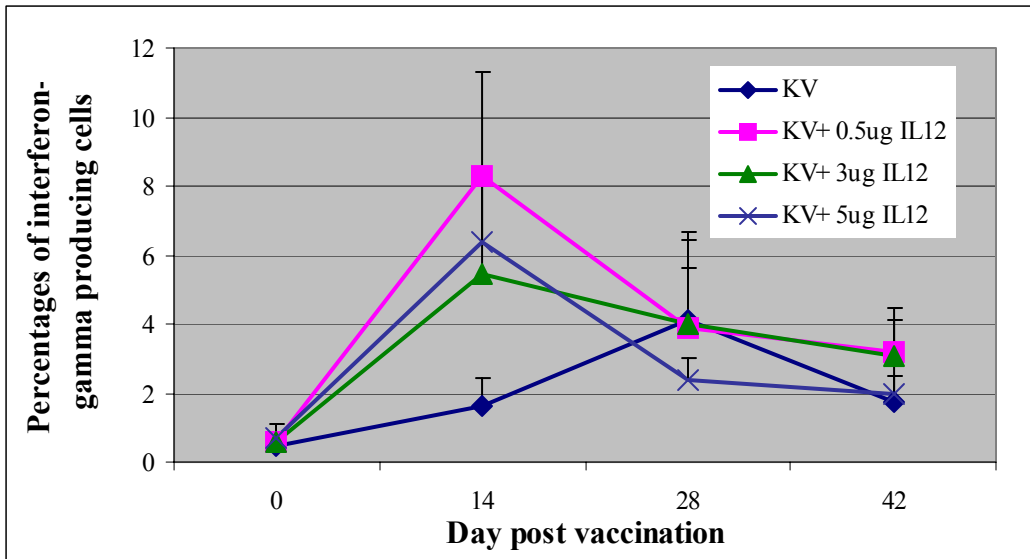


Figure 2. Cell-mediated immune responses determined by percentages of interferon-gamma producing cells in peripheral blood mononuclear cells in pigs vaccinated with a killed PRRSV vaccine containing variable concentrations of interleukin (IL)-12. Responses were expressed as mean percentages of interferon-gamma producing cells + one standard deviation.

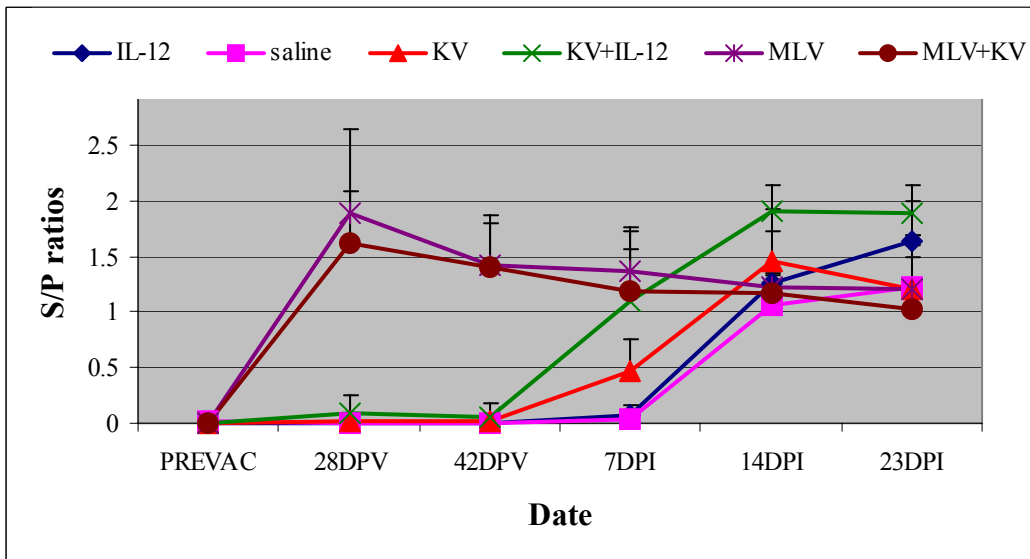


Figure 3. Antibody responses determined by IDEXX ELISA. Responses were expressed as mean of serum-to-positive (S/P) ratio + one standard deviation. An S/P ratio ≥ 0.4 is considered positive to PRRSV. IL-12 = interleukin-12; KV = killed PRRSV vaccine; MLV = modified live PRRSV vaccine.

Table 1.

Summary of average clinical respiratory scores of pigs at 0 – 9 days after inoculation with PRRSV.

Treatment groups ¹	Score ² at 0-9 day post infection
IL-12	6.4 ± 1.34 ^a
Saline	5.8 ± 1.30 ^a
KV	4 ± 1.34 ^b
KV + IL-12	1.73 ± 1.34 ^c
MLV	3.7 ± 1.77 ^b
MLV + KV	2.7 ± 1.77 ^{b,c}

Within each column, values with different superscripts are significantly different by least significant difference ($p < 0.05$).

¹ IL-12 = interleukin-12; KV = killed PRRSV vaccine; MLV = modified live PRRSV vaccine.

²Score: 0 = normal; 1 = mild dyspnea and/ or tachypnea when stressed; 2 = mild dyspnea and/ or tachypnea when at rest; 3 = moderate dyspnea and/ or tachypnea when stressed; 4 = moderate dyspnea and/ or tachypnea when at rest; 5 = severe dyspnea and/ or tachypnea when stressed; 6 = severe dyspnea and/ or tachypnea when at rest. Scores are displayed as mean ± one standard deviation

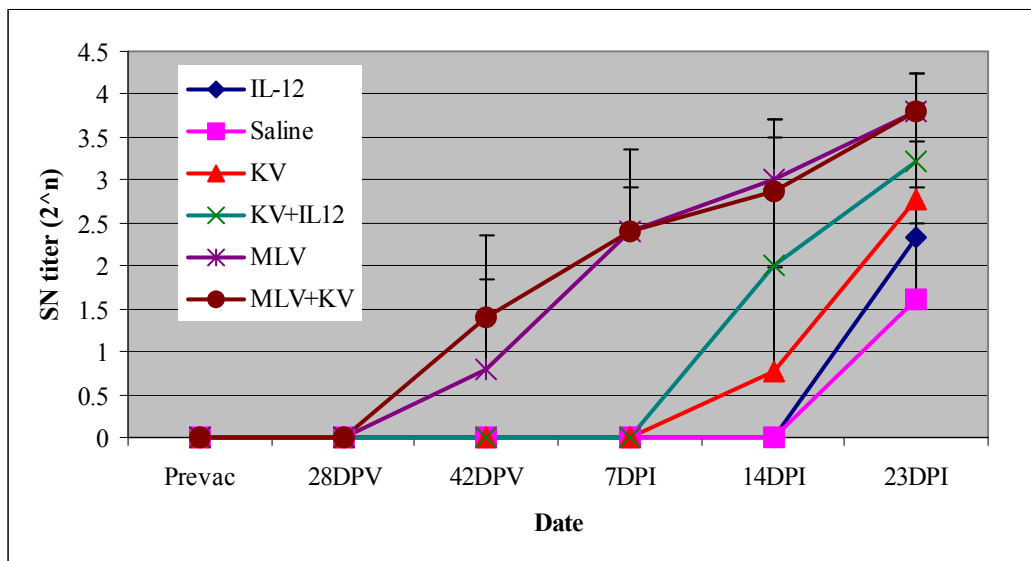


Figure 4. Serum neutralizing antibody responses. The assay was conducted using PRRSV strain ISU-P as the test antigen. Responses were expressed as the geometric mean titer + one standard deviation. IL-12 = interleukin-12; KV = killed PRRSV vaccine; MLV = modified live PRRSV vaccine.

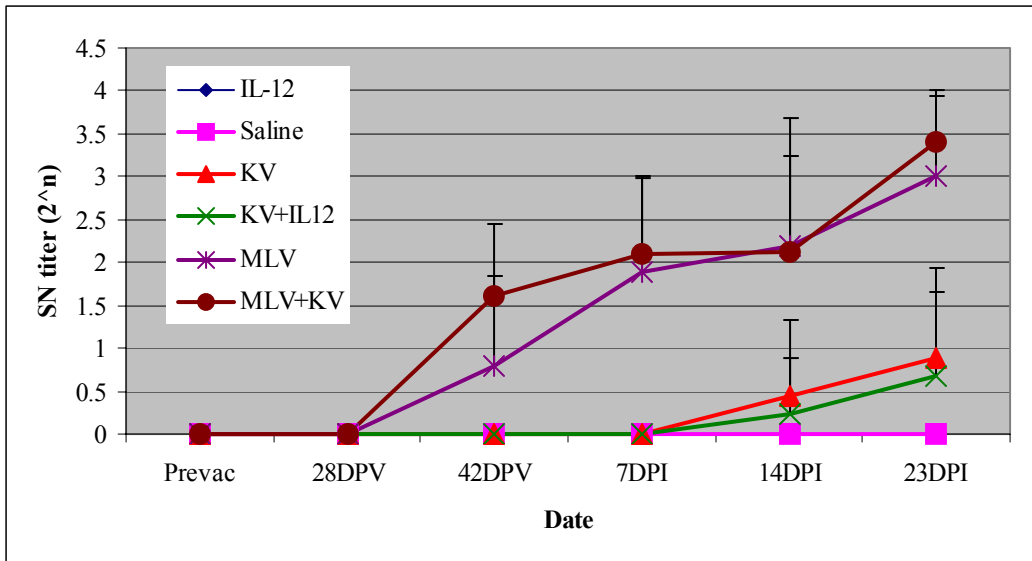


Figure 5. Serum neutralizing antibody responses. The assay was conducted using PRRSV strain VR-2332 as the test antigen. Responses were expressed as the geometric mean titer + one standard deviation. IL-12 = interleukin-12; KV = killed PRRSV vaccine; MLV = modified live PRRSV vaccine.