RESEARCH REPORT

SWINE HEALTH

**Title:** Effect of repeated PRRSV vaccination on lymphocyte response in sows
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**Abstract:**

The objective of this study was to investigate the effect on the immune system of long term exposure of sows to PRRSV from repeated vaccination with either modified live virus (MLV) or killed PRRSV vaccines. Serology and the response of specific populations of lymphocytes from peripheral blood to PRRSV antigens specific for each vaccine was measured. The study utilized cull sows obtained from PRRSV positive farms with a history of multiple vaccinations with one of the two vaccines and a PRRSV negative herd. Differences in antibody recall ability were detected between the two vaccine regimens by measuring serum antibody levels with both a commercial ELISA assay and a serum neutralization assay. The sows repeatedly vaccinated with the MLV vaccine failed to produce a recall antibody response upon re-vaccination with the MLV vaccine. Sows previously vaccinated with the killed vaccine demonstrated a slight increase in serum neutralizing antibodies only following vaccination with the MLV vaccine. In contrast, sows which received the killed vaccine and were boostered with the killed PRRSV vaccine demonstrated an increase in both types of antibodies. Statistical analysis of the lymphocyte proliferation assays is still underway. However, preliminary results suggest that the B cell response closely matches the antibody response. In addition, B cell proliferation appeared to occur independent of PRRSV strain used for stimulation. Preliminary analysis found that B lymphocytes from sows receiving the killed vaccine proliferated more than lymphocytes from sows repeatedly vaccinated with the MLV vaccine in response to revaccination. This study suggests that more information on the immune response induced by wild-type virus and repeated vaccination with a MLV vaccine is needed. The preliminary data in this study suggests

that repeated MLV PRRSV vaccination does not booster the immune response. However, challenge studies are required to determine the ability of a vaccine to induce protection against clinical disease.

**Introduction:**

Porcine reproductive and respiratory syndrome virus (PRRSV) remains one of the most important pathogens in the swine industry. Vaccination has emerged as a common tool for decreasing the impact of the virus on the breeding herd. However, little information is available on the effect of multiple vaccinations on the immune response of breeding animals and the practice of repeated vaccination with modified live virus (MLV) vaccine is controversial.

It has been demonstrated that sows develop protective immunity following PRRSV-induced reproductive losses based on observations that affected sows have normal litters despite the circulation of PRRSV within the breeding herd[1,2]. Lager et al. (1997) investigated the duration of immunity induced in sows by experimental exposure to PRRSV and subsequent challenge with the same virus and found protection lasted for at least 625 days[3]. In spite of the evidence of long term immunity induced by PRRSV, continued re-vaccination of sows is common. Sows are frequently vaccinated at 1 or 2 specific points (midgestation and/or lactation) during each parity[4]. The MLV vaccine currently on the market has been approved for use in healthy gilts and sows 6 to 8 weeks prior to breeding. Frequent vaccination with the MLV PRRSV vaccine is intended to provide greater protection against recurrence of reproductive failure. However, this strategy has often failed to produce stability in breeding herds where wild type virus continues to circulate resulting in infected offspring and sporadic PRRSV-induced reproductive failure. In addition, vaccine virus can circulate in the herd so serological testing for circulation of wild-type virus may be difficult to interpret. Finally, many sows which have been vaccinated multiple times over several years no longer have detectable antibodies against PRRSV. Approximately 2 years ago, a new killed PRRSV vaccine was approved for use midgestation. Accordingly, there is much less known about this vaccine compared to the MLV vaccine.

The importance of the loss of measurable antibodies in these older animals which have received multiple vaccinations over their lifetime with respect to herd susceptibility to PRRSV is unknown. One possible explanation for the loss of measurable circulating antibodies in pigs vaccinated multiple times is lymphocyte anergy or immune tolerance. ***We hypothesized that frequent exposure to MLV PRRSV vaccine and possibly field strains of PRRSV may lead to the development of lymphocyte tolerance or anergy resulting in the loss of immune responsiveness to PRRSV infection.*** This hypothesis was based on both field experiences and a preliminary study performed in conjunction with Dr. Butch Baker of PIC. In the preliminary study, it was found that sows repeatedly vaccinated with the MLV PRRSV vaccine did not respond serologically when re-vaccinated with the MLV vaccine independent of serological status. In contrast, when sows were vaccinated with the killed PRRSV vaccine, serum antibodies levels increased as detected with both the commerical ELISA assay and serum neutralizing assays. The results of this study in combination with field evidence suggested to us that repeated vaccination with the MLV PRRSV vaccine may result in the loss of ability of lymphocytes to respond to the vaccine antigens. The current MLV PRRSV vaccine infects the animal inducing an immune response similar to field infection but with minimal-to-mild clinical disease.

Studies performed in our laboratory demonstrated that pigs vaccinated once with RespPRRS® may exhibit mild clinical disease including lethargy, anorexia and fever. In addition, RespPRRS® vaccination induces generalized lymphadenopathy and mild pneumonia with PRRSV antigen detected in both alveolar and intravascular macrophages. These findings suggest that vaccination with a MLV PRRSV vaccine is similar in many respects to infection. In contrast, a killed vaccine, in theory, produces protective immunity without infection and may circumvent the impact of PRRSV on the immune system, in particular macrophages. It is important to recognize that the immune response induced by the two types of vaccines differ significantly and therefore the measured immune responses may also differ. MLV vaccination results in presentation of foreign antigens to the immune system primarily in the context of the class I major histocompatibility complex (MHC). This type of immune response potentially favors the development of a cellular type of immune response against intracellular pathogens. Conversely, a killed vaccine is ingested by professional antigen presenting cells, such as macrophages, and presented in the context of class II MHC resulting in primarily a humoral or antibody response. Although these are the classic scenarios expected with each of these types of vaccines, killed vaccines can induce cellular immune responses and MLV vaccines induce the production of antibodies. Therefore, the most efficacious type of vaccine used against each pathogen must be determined considering the pathogen, the immune response required for protection and potential side effects.

Possible explanations for the loss of immune responsiveness by sows following repeated vaccination include either lymphocyte anergy or tolerance. Anergy, which is a state of non-responsiveness to antigen, can occur with either T or B cells. Anergy can occur in B cells that chronically encounter and bind to abundant soluble antigen. When lymphocytes become anergic, they no longer respond to a specific antigen. In the case of B cells, this would result in no production of antibody following antigen stimulation. Tolerance is similar to anergy in that it also represents failure to respond to an antigen. Typically tolerance is induced to self proteins in the body to prevent the immune system from reacting against itself. The interaction between lymphocytes and viral antigens presented by antigen presenting cells, such as macrophages, is influenced by both a signal through the antigen specific receptor and an interaction between pairs of accessory molecules on the lymphocyte surface. If either of these signals are missing, no immune response is initiated. A potential mechanism for induction of tolerance by PRRSV is that the virus infection of macrophages induces a down regulation of the signaling mechanisms for lymphocyte activation. Accordingly, this would result in a failed immune response to PRRSV antigen.

We speculate that the loss of immune responsiveness to PRRSV may play a role in the resurgence of PRRSV outbreaks observed in some herds. This study investigated the immune responses against PRRSV antigens from both MLV and killed vaccine in sows from farms with histories of multiple vaccination of sows with either product. Sows on both farms have been documented to be both positive and negative for antibodies against PRRSV. Both herds from which the vaccinated sows were procured were positive for wild-type virus. Sows from a PRRSV negative herd were used as controls for both vaccine response and nonspecific lymphocyte responses. This study assessed the immune response to PRRSV by measuring both serum antibodies and the ability of specific populations of lymphocytes to respond to PRRSV antigens specific for each vaccine. Both neutralizing and non-neutralizing antibodies

were measured as well.

**Objectives:**

Our overall objective is to identify the effects of PRRSV on the immune system. We proposed to investigate the effects of long-term exposure to PRRSV from repeated PRRSV vaccination by measuring the responsiveness of lymphocytes in sows. Therefore, we investigated the responsiveness of the lymphocytes to both homologous and heterologous strains of PRRSV following vaccination. In addition, we evaluated the serological responses induced by each vaccine.

**Procedures:**

*Animals:* Cull sows from 3 herds were used in the study. Herd 1 sows (MLV) were procured from a herd with a long history of vaccination with one MLV vaccine (RespPRRS®, Boehringer Ingelheim/NOBL Labs). The herd was diagnosed as a clinically PRRSV positive herd in 1993 and has been on a 6/60 (6 days after farrowing, 60 days after breeding) vaccination program since the vaccine was released. Sows on this farm have been documented to be both positive and negative for antibodies against PRRSV. Sows from Herd 2 (KV) had received a minimum of 4 vaccinations with a commercial killed PRRSV vaccine (PRRomiSe™, Bayer Animal Health). This herd has been historically PRRSV positive and had a confirmed PRRSV outbreak the spring of 1999. Herd 3 (NEG) has been documented to be seronegative for PRRSV and the sows were used as vaccine controls as well as procedural controls for the immune responsiveness of lymphocytes to PRRSV.

Twelve sows from each herd were obtained and randomly assigned to one of 3 vaccine groups: i) MLV PRRSV vaccine; ii) killed PRRSV vaccine; and iii) no vaccine. Sows were vaccinated with the first dose of killed vaccine approximately 2 weeks following arrival at ISU which allowed time for acclimation. A second dose of killed vaccine was administered 2 weeks after the first. A single dose of the MLV PRRSV vaccine was administered to the appropriate group at the same time as the second killed vaccine was administered. Heparinized blood and serum was collected from all sows prior to the first vaccination, at the time of the second killed vaccine and first MLV vaccine, and 7 and 17 days following the final vaccination.

*Virus:* PRRSV strains 2332 and ISU-P , the parent virus strains for the MLV and killed vaccines respectively, were grown on MARC cells and purified by sucrose gradient as previously described[5]. The purified virus was used to stimulate lymphocytes *in vitro*.

*Lymphocyte proliferation:* Heparinized blood was obtained at the time intervals described above. Lymphocyte isolation was performed using differential centrifugation as previously described[6]. Lymphocytes were stained with a dye, PKH, and incubated with either Concanavalin A (Con A) to confirm the responsiveness of the lymphocytes, or one of the two strains of PRRSV antigen. Lymphocytes from all sows were cultured with both antigens to measure cross-reactivity between the virus strains. Cells were cultured for 5 days with either media or the appropriate antigens outlined above. Flow cytometric analysis was used to measure both proliferation and population of lymphocytes which may have proliferated in response to each of the antigens.

*Serology:* A commercial PRRSV ELISA (IDEXX) was used to measure non-neutralizing antibodies to PRRSV. Measurement of serum neutralizing antibodies (SN) was done by South Dakota State University at Brookings under the supervision of Dr. Eric Nelson.

*Statistical analysis:* Analysis of variance was used to detect significant differences

among groups. A non-parametric ANOVA (Kruskal-Wallis) was used for non-normally distributed data or when group variances were dissimilar.

**Results:**

The objective of this study was to measure the effect of long term exposure of the immune system to PRRSV. In order to accomplish this goal, cull sows from three different sources with differing vaccination protocols and health status were used. Statistical analysis of this study is still ongoing. This is due to the complexity and large amount of data collected in this study.

Serum antibody results are summarized in Table 1. The sows in Herd 1 (MLV) which were vaccinated with the killed vaccine demonstrated increased antibody levels over the course of the study as measured by the commercial ELISA, but no increase in SN levels were detected. There was no increase in antibody levels in either assay following re-vaccination with the MLV vaccine in Herd 1 sows. Sows in Herd 2 (KV), previously vaccinated with the killed product, demonstrated an increase in both types of antibodies following re-vaccination with the killed vaccine. In contrast, KV sows receiving the MLV vaccine demonstrated no increase in antibodies measured by ELISA and only a slight increase in SN antibody levels. These sows would have received the MLV product as young gilts during acclimation and it is unknown what effect the earlier vaccination had on the immune response observed in this trial. The anamnestic or recall immune response appeared to be minimal. As expected, there was no increase in antibody levels in sows in Herds 1 and 2 which were non-vaccinated. Sows from the negative herd (NEG), vaccinated with the MLV vaccine demonstrated increased antibody levels as measured by the commercial ELISA, but no increase in SN antibody levels. No seroconversion was observed by either assay in the NEG sows vaccinated with the killed product. Non-vaccinated NEG sows remained seronegative throughout the course of the trial. The results of this study were consistent with an earlier study which found that sows from Herd 1 which had previously been vaccinated with the MLV vaccine did not show any increase in either type of antibody following re-vaccination with the MLV product.

Data analysis of the lymphocyte stimulation assays is still ongoing. Due to the large number of variables measured in this study, the statistical analysis has proven to be extremely complex. In addition, the small number of animals in each group (n=3 or 4) will further complicate statistical analysis in order to determine significance. The PKH assay used in this study is unique as it allows us to ascertain the specific populations of lymphocytes proliferating in response to an antigen, in this case PRRSV. This aids in our ability to differentiate a T cell response (cell mediated immune response) from a humoral response induced by B cells.

Differences were observed between the groups in the number of B lymphocytes stimulated by antigen. B cells appeared to respond to both strains of PRRSV by the sows in a similar manner suggesting the response to PRRSV is independent of strain of virus. B cells from sows in Herd 2 (KV) re-vaccinated with killed vaccine showed the greatest response to PRRSV stimulation in culture independent of virus strain. Sows in all groups showed some B cell stimulation in response to the MLV PRRSV vaccine, although the response was lower than to the killed vaccine. Sows from the PRRSV negative herd showed no stimulation to the killed vaccine. These results are consistent with the antibody response. Herd 1 and 2 sows which received the killed vaccine had higher antibody responses following vaccination than the groups receiving the MLV

vaccine. The response to the MLV vaccine was greater in the sows from Herd 2 (KV) and the NEG sows compared to the sows which had received previous repeated MLV vaccinations. Again this was observed with both strains of virus used to stimulate the lymphocytes *in vitro*. These results suggest some cross protection between strains of PRRSV. Further statistical analysis is being preformed to ascertain any statistical differences between herds and vaccine status.

T cell analysis demonstrated no apparent differences between the PRRSV strains by among the vaccine groups. However, more complete statistical analysis is still ongoing and may demonstrate subtle differences between groups.

In conclusion, this study confirmed that the immune response induced by the MLV PRRSV vaccine is different from that of a killed product. Both the current study and the earlier preliminary study performed in conjunction with Dr. Butch Baker which contrast the immune responses induced by MLV and killed PRRSV vaccines suggest that repeated vaccination with the MLV vaccine does not booster the immune response. This lack of an anamnestic or recall response may confirm our hypothesis that repeated chronic exposure to PRRSV antigen through vaccination may diminish the ability of lymphocytes to respond to the virus. However, this study did not conclusively demonstrate the lack of response by lymphocytes to PRRSV in the MLV vaccinated herd (Herd 1). The efficacy of a vaccine cannot be determined by measurement of an immune response only. Challenge studies are required to determine the ability of a vaccine to induce protection against clinical disease. This study did not use challenge as a basis of determining the response to the vaccines. The results of this study highlight the need for increased information on the immune response induced by chronic exposure to wild-type virus and repeated vaccination. Increased understanding of the immune response induced by repeated vaccination is required for not only PRRSV, but other pathogens in order to develop the appropriate intervention strategies needed to control disease in breeding herds where animals can be kept as long as 4-5 years.

**References**


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Table 1. Serology data as measured by either a commercial ELISA (IDEXX) or serum neutralizing antibody assay from sows obtained from herds previously seronegative or vaccinated with MLVor a commercial killed PRRSV vaccines. Sows under study were either vaccinated with MLV or killed PRRSV vaccines or received no vaccine.

| | | | Commercial ELISA | | | | Serum Neutralizing Assay | | |
|---|---|---|---|---|---|---|---|---|---|
| Source | Vaccine | Arrival | Vacc 1* | Vacc 2 | Vacc 3 | Diff** | SN1[§] | SN2 | SN Diff |
| Herd 1 (MLV) | MLV[£] | 1.551 | 1.280 | 1.146 | 1.215 | -0.337 | 5.25 | 4.25 | -1 |
| | KV[ƒ] | 0.925 | 1.033 | 1.401 | 1.328 | 0.403 | 3.75 | 3 | -0.75 |
| | None | 1.204 | 0.819 | 0.708 | 0.505 | -0.699 | 3.25 | 2.75 | -0.5 |
| Herd 2 (KV) | MLV | 0.481 | 0.311 | 0.386 | 0.248 | -0.234 | 1.5 | 1.75 | 0.25 |
| | KV | 0.196 | 0.714 | 1.055 | 1.005 | 0.809 | 1.5 | 3.5 | 2 |
| | None | 0.374 | 0.317 | 0.293 | 0.256 | -0.188 | 0.67 | 0.67 | 0 |
| Herd 2 (NEG) | MLV- | 0.033 | 0.067 | 0.111 | 0.892 | 0.924 | 0 | 0 | 0 |
| | KV | 0.001 | 0.035 | 0.021 | 0.001 | 0.000 | 0 | 0 | 0 |
| | None | -0.041 | 0.161 | 0.002 | -0.002 | 0.038 | 0 | 0 | 0 |

* Serology expressed as S/P ratios. Vacc 1 = time of 1st vaccination; Vacc 2 = time of 2nd vaccination; Vacc 3 = 17 days post 2nd vaccination

** Diff = Difference in S/P ratio from arrival to Vacc 3.

§ SN = Serum neutralizing antibodies expressed as a log base 10. SN1 = arrival; SN2 = 17 days post vaccination.

£ MLV = Modified live virus PRRSV vaccine

ƒ KV = Killed PRRSV vaccine