RESEARCH REPORT
SWINE HEALTH

Title: Protective Immunity against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Obtained by Passive Administration of Antibodies: Optimization of the Conditions - **NPB# 02-033**

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Abstract: Previous work had demonstrated that transfer of antibodies highly enriched in neutralizing activity to PRRSV protected pregnant sows against reproductive failure and conferred sterilizing immunity in sows and offspring. To study these conditions using a young pig PRRSV challenge model, we used two-week-old piglets as recipients of antibodies injected by the intraperitoneal route, prior to intranasal challenge with PRRSV. Piglets receiving an amount of neutralizing antibodies sufficient to reach a serum titer of 8 consistently did not develop viremia (systemic PRRSV in blood), Importantly, piglets receiving early PI, non-neutralizing antibodies (obtained from serum of infected pigs at≤ 21 days post-infection) developed viremia similarly to the infected control group, which indicates that protection against viremia is specifically associated to the neutralizing antibodies. In spite of the protection of the neutralizing antibody-transferred animals against viremia, their lungs, tonsils and peripheral lymph-nodes contained replicating PRRSV at the same level as the infection control group. In addition, these animals excreted infectious virus to sentinels at the same rate as the infection control animals. Thus, the presence of anti-PRRSV neutralizing antibodies in serum with a titer of the 8 is enough to hamper viremia but not peripheral tissue seeding and transmission to contact animals. In addition, these experiments cast doubt about the significance that viremia may have in pathogenesis of PRRSV infections.

Introduction: The unraveling of the mechanisms of PRRSV protective immunity is a top priority for the NPB grant program. Although the existence of PRRSV-specific protective immunity is recognized, the molecules or cells that mediate this protection have not been clearly identified. Contrasting opinions existed about the protective significance of antibodies in PRRSV infections, specifically about the significance of the PRRSV-specific neutralizing antibodies (Definition of neutralizing antibody: a type of antibody that, when mixed in a test tube with PRRSV, inactivates it by loss of virus infectivity). Many laboratories reported them as ineffective in the response to PRRSV, while other reports seemed to suggest that protection mediated by antibodies against PRRSV indeed exists. On this basis, we decided to study the role of antibodies in protection against PRRSV. Our previous experiments provided unequivocal evidence that PRRSV-neutralizing antibodies alone can fully prevent the transplacental infection with PRRSV and completely extinguish the infection of PRRSV in pregnant females. In this project we proposed to use a model PRRSV challenge in young pigs that would permit us to study the optimal conditions of neutralization in vivo and efficacy of antibodies to provide protective immunity.

Objectives:
1) To know the minimal end-point of serum-neutralizing (SN) titer in the recipient animal that would protect it against PRRSV challenge,
2) To ascertain the ability of neutralizing Igs to protect against a heterologous PRRSV strain and
3) To know if, besides preventing the infection, PRRSV antibodies can “treat” or “cure” an already established infection.

Materials & Methods: Hyperimmune serum containing high titer of PRRSV-neutralizing antibodies was obtained by inoculation of 3-month old pigs with a highly pathogenic PRRSV(designated here as homologous virus) followed with repeated inoculations of the same strain in conjunction with complete and incomplete Freund’s adjuvant interspersed in periods of one and a half months. After the neutralizing titer of antibodies reached 1:32 or higher, the animals were killed and the total serum of each animal was collected. Pool of the serums was used to precipitate the IgG (immunoglobulins), purify and quantitate. The solution of PRRSV-neutralizing immunoglobulins was used for passive transfer experiments that involved the intraperitoneal inoculation of two-week-old piglets to obtain groups of animals with different PRRSV-neutralizing titers in the serum, ranging from 1:4 to 1:32 after 24 hs post-injection. The animals so passively transferred were immediately challenged with homologous PRRSV by the intranasal route and the following parameters were evaluated for each group: temperature, viremia (at 4 and 7 days PI), infectious PRRSV load in diverse tissues (lung, tonsils, several lymph nodes) at 14 days PI (necropsy time), and transmission of infection to sentinels by each group for the total PI period (14 days). One group of animals was transferred with early PRRSV-specific immunoglobulins (collected within the first 21 days PI) which are known to be highly reactive with PRRSV but that do not neutralize the virus.

Results: Pigs receiving neutralizing antibodies at 1:8 or higher titer exhibited a reduction in the rectal temperature peak after challenge with PRRSV (data not shown). Pigs receiving an amount of neutralizing antibodies sufficient to reach a serum titer of 8 or higher consistently did not develop viremia (systemic PRRSV in blood) (table 1 and figure 1). Importantly, pigs receiving early PI, non-neutralizing antibodies (obtained from serum of infected pigs at≤ 21 days post-infection) developed viremia similarly to the

infected control group (figure 1), which indicates that protection against viremia is specifically associated to the PRRSV-neutralizing antibodies but not to the non-neutralizing early post-infection antibodies. In spite of the protection of the neutralizing antibody-transferred animals against viremia, their lungs, tonsils and peripheral lymph-nodes contained replicating PRRSV at the same level as the infection control group (examples of tonsil titers are shown in Figure 2). In addition, these animals excreted infectious virus and infected sentinels at the same rate as the infection control animals (Table 1). An additional experiment with animals that had received the highest possible titer obtainable by passive titer (1:32) confirmed that the viremia was completely precluded , and that in at least some of the animals it was possible to obtain sterilizing immunity , as evidenced by absence of viral load in tissues at 14 day PI in 3 of the animals(figure 3). Nevertheless the groups infected sentinels (table 1), which is consistent with the notion that some of the animals had sizable viral load in peripheral tissues (figure 3).

Discussion: The passive transfer experiments described in this report led us to several conclusions:

1. Only Neutralizing Antibodies Are Effective in Clearing Viremia and “Protecting” Pigs

This observation is important as it confirms that any protective effect of the passive transfer of immunoglobulins (i.e. clearance of viremia, reduction of temperature) would be related to the neutralizing antibody fraction and that the early antibodies that compose the seroconversion to PRRSV detectable by use of the commercial ELISA kits, which are non-neutralizing antibodies, are not responsible for protection.

2. Transfer of PRRSV-Neutralizing Antibodies May Mitigate Clinical Symptoms but Does Not Clear Infection in Young Weaned Pigs

In animals that had received neutralizing antibodies at a titer of 8 or higher it was observed a reduction of the rectal temperature and a clearance of the viremia. It should be noted, however, that the presence of anti-PRRSV neutralizing antibodies in serum at a titer of 8 may be enough to hamper viremia but not to prevent peripheral tissue seeding and transmission to contact animals. Under conditions of maximal concentration of neutralizing antibodies attainable by passive transfer in our experimental system (1:32, figure 3), sterilizing immunity was apparent in at least 3/6 animals, which would suggest that the full protective effect may be related to a threshold of replicating virus to be overcome by antibodies in young animals. Such threshold could be higher in young pigs than in sows, in which sterilizing immunity was attained in the totality of individuals at lower concentrations of neutralizing antibody transfer.

3. Viremia does not Appear to be Essential for Viral Systemic Spread and Transmission in Young Pigs

It is remarkable that animals in which viremia had been blocked had a normal dissemination of infection to peripheral tissues, and, furthermore, that they were able to shed infectious PRRSV and infect sentinels. It should be noted that the animals were nonviremic based on lack of detection of either infectious PRRSV or PCR signal in serum. However, PCR analysis of peripheral blood mononuclear cells of these animals gave a moderate but detectable positive signal(data not shown) , thus indicating that the dissemination may take place in macrophages of blood as well a lymph, in spite of the

absence of extra cellular(serum) viremia. It is important to bear in mind, then, that absence of detectable viremia is not an indicator of negative PRRSV infection status.

4. Pathogenesis and Kinetics of PRRSV Infection Appears to be Different in Young Pigs or in Adults (possible reasons: Macrophage Permissiveness?)

The inability of high concentrations of neutralizing antibodies to preclude infection of PRRSV in young pigs may indicate that in this type of animals the level of replication in macrophages may be significantly higher than in adult animals (i.e., sows). From our previous experiments it was evident that passive transfer of neutralizing antibodies successfully stopped viremia, thereby precluding the seeding of placenta and the transfer of infection to offspring.

Lay Interpretation: Neutralizing antibodies are those antibodies produced by a pig infected with PRRSV which, when mixed with PRRSV in a test tube, render it uninfectious by inactivation. Based on the results of previous experiments involving pregnant sows, we know that PRRSV neutralizing–antibodies may completely protect a sow against the infection with this virus. However, the results from this NPB project indicate that mechanisms of protective immunity mediated by these antibodies against PRRSV are different when comparing adult animals (pregnant sows) and young pigs. While a relatively low level of neutralizing antibodies in blood may fully protect sows against PRRSV-induced abortion, the amount of neutralizing antibodies necessary to obtain full protection of a young weaned pig against PRRSV generalized infection would be significantly higher. This inability of neutralizing antibodies to fully protect young pigs probably reflects differences in the level of multiplication of PRRSV in the body of young pigs and in adult animals. A young pig is known to contain cells that are significantly more sensitive to PRRSV than those of an adult animal such as a pregnant sow.This research indicates that protection by vaccines may be simpler to obtain in sows than in young pigs. While vaccination with a commercial vaccine may be effective against abortions, immunizing young, weaned pigs with the same vaccine would still remain more challenging. Important additional information obtained in this NPB project is that the measurement of viremia (PRRSV in blood) is not a trustable indicator of the infection status of the animal. We observed that an animal may be non-viremic but still replicate the virus abundantly in their tissues, efficiently transmitting the PRRSV infection to contact animals. For further information please contact: Fernando A. Osorio, University of Nebraska-Lincoln, fosorio@unl.edu, phone: 402-472-7809

SN Protective Titer	Viremia	Transmission to sentinels	
		1-7 dpi	7-14 dpi
1:4	+	+	+
1:8	neg	+	+
1:16	neg	ND	ND
1:32	neg	-	+

Table 1 Results of viremia (PRRSV in blood) and transmission to sentinels by groups of animals onto which had been passively transferred PRRSV neutralizing immunoglobulins at different concentrations. The titers shown under " SN Protective Titer" refer to the endpoint serum-neutralization titer in the blood of the animals 24 hs after the passive transfer of immunoglobulins and immediately prior to the challenge with infectious PRRSV. The "Transmission to Sentinels" columns include the results of transmission to sentinel animals that were put in contact with the infected groups for a week, then removed, and replaced with a new set of sentinels that were maintained in contact with the group for another 7 days, after which time the second pair of sentinels was removed. Upon removal, the sentinels for 1st and second week of contact were maintained for additional 15 days looking for signs of PRRSV infection.

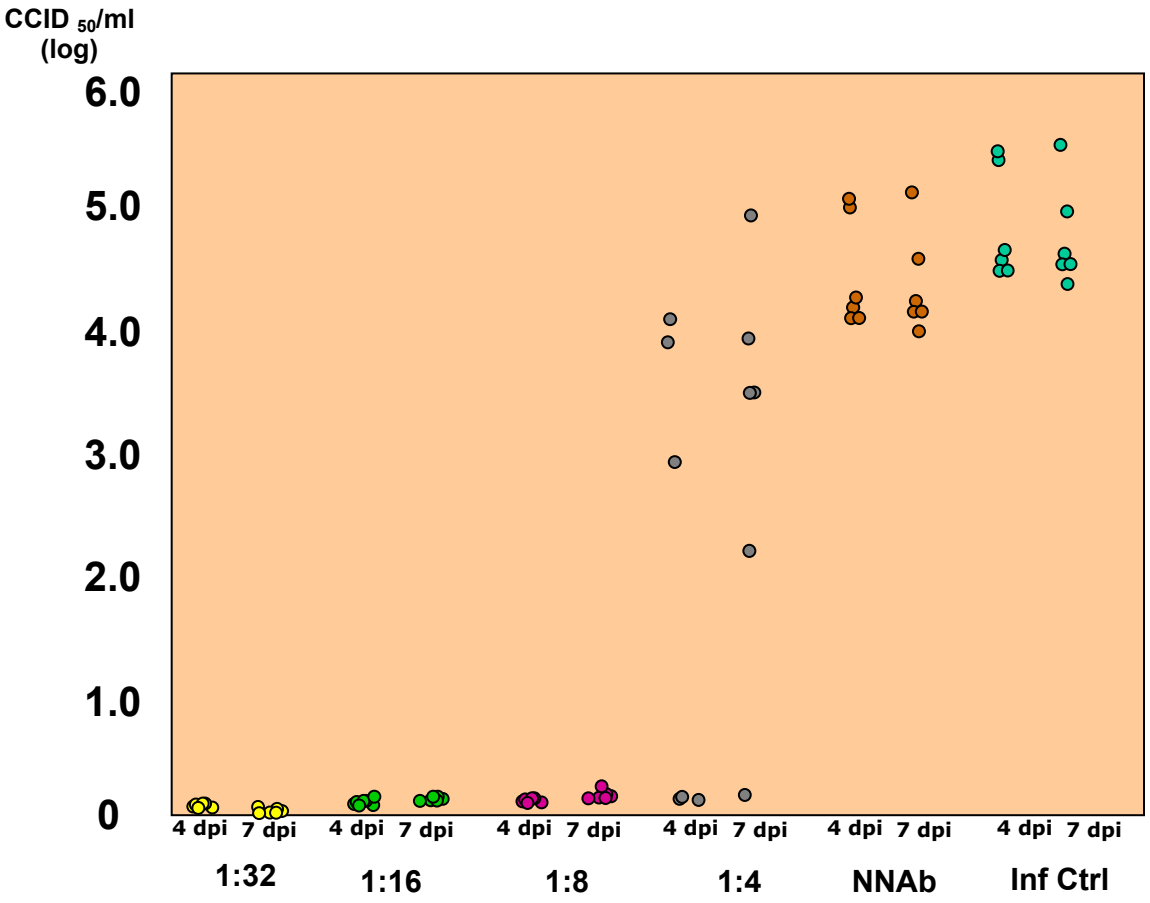

Figure 1: Infectious titer in blood of different groups of pigs passively injected with different concentrations (1:4 to 1:32) of PRRSV-neutralizing antibodies or non-neutralizing early PI antibodies (NNAb**).** Inf Ctrl: group that did not receive passive antibodies

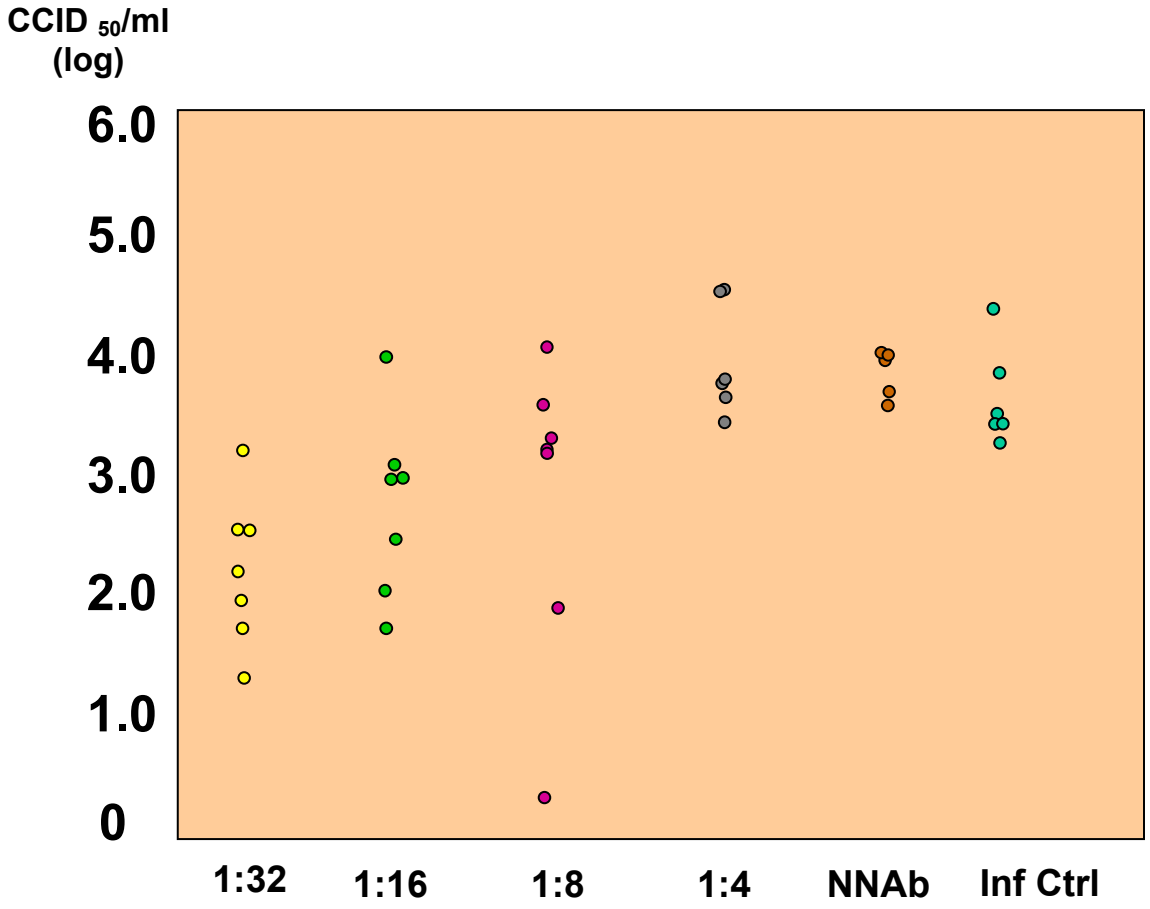

Figure 2: Viral load (infectious titer per gram of tissue) in tonsils of the same groups of animals shown in figure 1.

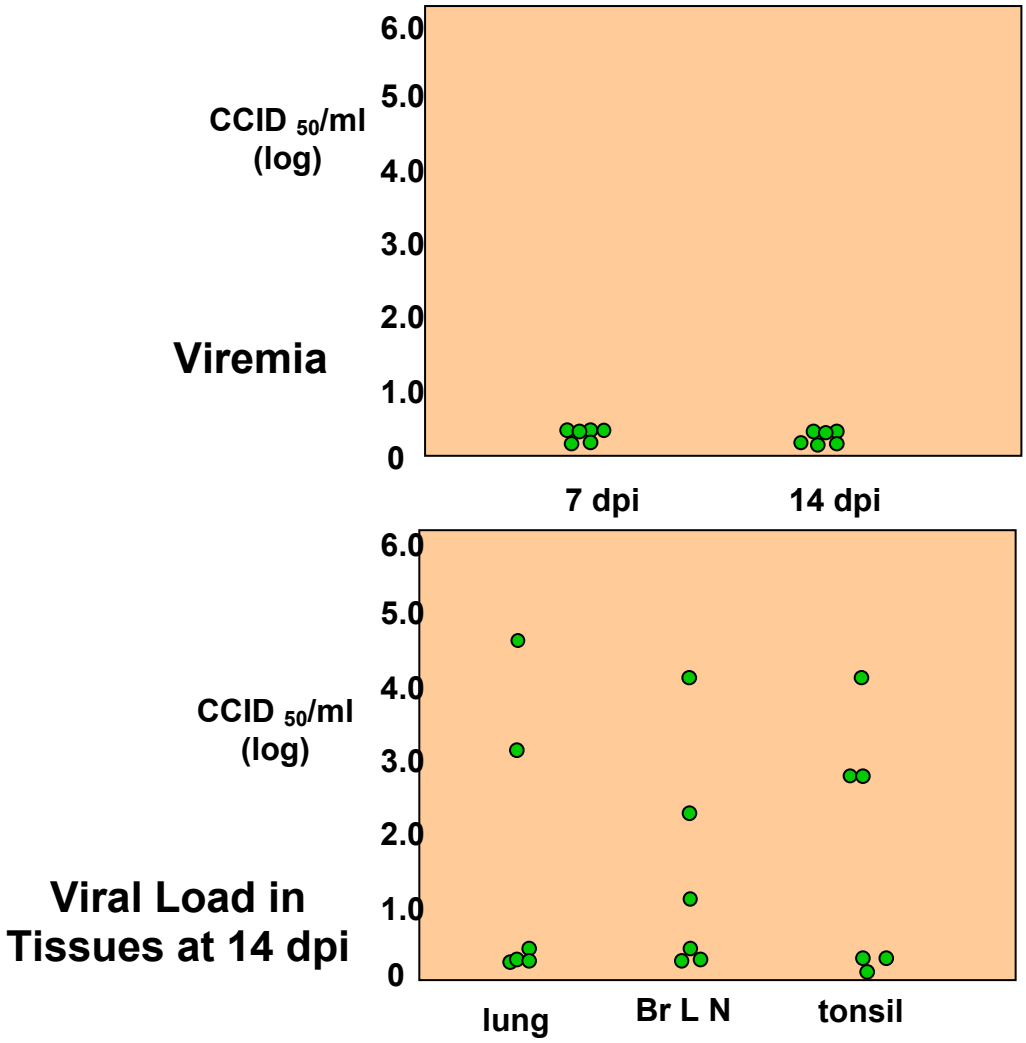

Figure 3 Viremia and viral load observed in a group of animals that have been infected with PRRSV after receiving a passive transfer of PRRSV-neutralizing antibodies that established in the animals a blood SN titer of 32.The animals in the group were put in contact with sentinels. The group shed virus to the second week sentinels but not during the first week of contact (see Table 1)