

# Passive Immunization of Piglets Using Equine Plasma Containing PRRS Virus-Neutralizing Antibodies

## A.S Leaflet R2255

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### Summary and Implications

Horses were inoculated with several strains of virulent PRRS virus. Sera from the horses were tested for the presence of PRRS virus-neutralizing antibodies. Large volumes of equine plasma were collected and used to passively immunize piglets. Sera were collected at various time points after immunization and tested for virus-neutralizing activity.

These results show that horses are capable of generating high neutralizing antibody titers to PRRSV when exposed to virulent virus. Piglets develop neutralizing antibody titers to PRRSV when passively immunized with a sufficient volume of  $\alpha$ -PRRSV equine plasma.

### Introduction

Previous studies have indicated that passive immunization of pigs with hyperimmune swine plasma can confer complete or partial protection to virulent PRRSV challenge, as determined by clinical signs and viremia. While horses historically have been used to raise large volumes of antibody to different organisms and toxins, there is currently no published data regarding the inoculation of horses with live PRRSV. The objective of our research was to produce high-titer antiserum in horses which could be used to passively immunize pigs for the prevention of PRRS clinical signs and viremia.

### Materials and Methods

#### *Immunization of Horses with PRRSV*

Each horse received a total of five intramuscular injections of virulent PRRSV. The same strain was used in the first two injections, while three different strains were used for subsequent injections. Each inoculation consisted of a 10 ml volume split between three injection sites. All strains were sequenced and titrated at  $10^5$  TCID-50 per ml. Horses were inoculated on Days 0, 21, 41, 84, and 126, and study termination occurred on Day 137.

#### *Concentration of Equine Plasma*

Plasma collected at study termination was put into lengths of dialysis tubing (12-14,000 MWCO) and covered with 40,000 MW polyethylene glycol. After 3.75 hours the concentrated plasma was collected and combined with raw plasma, resulting in a final

concentration of 3.8x. The same procedure was used on normal horse plasma. All plasma was sterilized through 0.45  $\mu$ m filters.

#### *Passive Immunization Studies*

Study I utilized filter-sterilized equine plasma (normal and  $\alpha$ -PRRSV). Eight pigs were each injected (sub-Q) with 80 ml of  $\alpha$ -PRRSV equine plasma, and four pigs were each injected (sub-Q) with 80 ml of normal equine plasma. Blood was collected at various times following immunization and tested to determine circulating virus-neutralizing antibody (FFN) titers.

Study II utilized the concentrated equine plasma (normal and  $\alpha$ -PRRSV). Six pigs received concentrated  $\alpha$ -PRRSV plasma at a rate of 10 ml per kg bodyweight. Two pigs received a dose of 5 ml per kg bodyweight. Four pigs received concentrated normal equine plasma at a rate of 7.5 ml per kg bodyweight. Blood collection and testing methods were the same as Study I.

### Results

#### *Immunization of Horses with PRRSV*

Horses did not become febrile or show any clinical signs of disease throughout the study. No injection site reactions were observed. No detectable virus was present in serum when tested on MARC-145 cells. Both horses had an initial FFN titer of  $<1:4$  against the first strain used. At study termination on Day 137, the horses had a geometric mean FFN titer of  $1:181$  against the first strain used. The geometric mean FFN titers against the 3rd and 4th strains used were  $1:128$  and  $1:90$  respectively.

These results are detailed in Figure 1.

#### *Concentration of Equine Plasma*

The  $\alpha$ -PRRSV equine plasma used in the concentration procedure had a beginning FFN titer of  $1:256$ . Following concentration to 3.8x the FFN titer increased to  $1:1024$ . The normal equine plasma used in the concentration procedure had a beginning titer of  $<1:4$ . Following concentration to 3.4x the FFN titer increased to  $1:8$ .

These results are detailed in Figure 2.

#### *Passive Immunization Studies*

In Study I, piglets ( $n=8$ ) that received  $\alpha$ -PRRSV equine plasma had a geometric mean FFN titer of  $1:6$  at 24 hours post immunization. Piglets ( $n=8$ ) that received normal equine plasma had a geometric mean FFN titer of  $<1:4$  at 24 hours post immunization.

In Study II, piglets (n=2) that received concentrated  $\alpha$ -PRRSV equine plasma at a rate of 5 ml/kg had a geometric mean FFN titer of 1:16 at 24 hours post immunization. Piglets (n=6) that received the same plasma at a rate of 10 ml/kg had a geometric mean FFN titer of 1:25 at 24 hours post immunization. Piglets (n=4) that received concentrated normal equine plasma at a rate of 7.5 ml/kg had a geometric mean FFN titer of <1:4 at 24 hours post immunization.

Results of passive immunization studies are detailed in Figure 3.

### Discussion

These results indicate that horses are capable of generating relatively high virus-neutralizing antibody titers when immunized with virulent PRRS virus. Equine plasma can be concentrated and used to passively immunize piglets, which results in circulating virus-neutralizing antibody titers at 24 hours post-immunization.

One benefit of using equine plasma is the comparatively large volume of plasma that can be recovered from a single animal. Another benefit is that the current PRRS ELISA diagnostic test is specific to porcine  $\alpha$ -PRRSV antibodies, which allows piglets immunized with equine plasma to still show a negative IDEXX PRRS ELISA result. In this way, the IDEXX PRRS ELISA can be used to determine viral exposure in pigs that receive passively administered equine antibodies.

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Strain	Mean FFN Titer	Horse #1 FFN Titer	Horse #2 FFN Titer
1 <sup>st</sup>	1:181	1:128	1:256
2 <sup>nd</sup>	ND	ND	ND
3 <sup>rd</sup>	1:128	1:64	1:256
4 <sup>th</sup>	1:90	1:64	1:128

Figure 1. FFN titers (Day 137) to strains of PRRSV used in the immunization of horses.

	Not concentrated (Study I)	Concentrated (Study II)
$\alpha$ -PRRSV equine plasma	1:256	1:1024
Normal equine plasma	<1:4	1:8

Figure 2. FFN titers (1<sup>st</sup> strain) of equine plasma used to passively immunize piglets.

Study	Dosage Rate	Type of Plasma	Mean FFN Titer @ 24 hr post imm.
I	80 ml/pig	$\alpha$ -PRRSV	1:6
I	80 ml/pig	normal	<1:4
II	5 ml/kg	Conc. $\alpha$ -PRRSV	1:16
II	10 ml/kg	Conc. $\alpha$ -PRRSV	1:25
II	7.5 ml/kg	Conc. normal	<1:4

Figure 3. FFN titers (1<sup>st</sup> strain) at 24 hours post-immunization of piglets receiving different types and dosages of plasma in passive immunization studies.