Characterization of Broadly Neutralizing Antibodies to PRRSV

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- PRRSV antibodies were determined to be broadly binding, but only homologously neutralizing, suggesting a polyclonal response, involving multiple PRRSV specific antibodies might be necessary for PRRSV neutralization. This means that vaccinations aiming to protect from infection likely need to involve exposure to multiple strains of PRRSV to induce a broad protective immunity, rather than relying on single-strain vaccines.

Background:
How the pig neutralizes PRRSV is unknown. Whether it elucidates a polyclonal (multiple) or monoclonal (single) antibody response, what parts of the virus (viral epitopes) are involved in neutralization, and how broadly neutralizing antibodies are generated are just a few of many unanswered questions. Some pigs can develop neutralizing antibodies against PRRSV weeks after exposure, protecting the pig from developing clinical disease and from shedding virus.[1] Development and quality of neutralizing antibodies varies from pig to pig, with some pigs developing a strong response, some developing no response, and, in many pigs, a response somewhere in between the two, despite all pigs having similar vaccine or field virus exposure.[2] The cause for these differences in the immune response to PRRSV is unknown. Rare individuals develop cross-protective or broadly neutralizing antibodies, giving them the ability to effectively neutralize virus they have had no prior exposure to and protecting themselves from future novel PRRSV exposures.[3,4]
Here, we examine the antibodies of pigs who show evidence of broad neutralization against PRRSV and identify PRRSV-binding B cells and the antibodies they produce to begin to determine the qualities of antibodies that could potentially lead to broad viral neutralization.

Materials and methods:
B cells were extracted and immortalized (so the cells can be grown indefinitely in a laboratory) from the blood of two pigs (BNW4 and BNW7) that show evidence of broad neutralization against both PRRS type 1 and PRRS type 2 viruses. Antibodies produced by these B cells were examined for their ability to bind and neutralize a variety of PRRS viral isolates and to bind various PRRSV proteins.

Results:
Five unique PRRSV-specific, antibody-secreting immortalized B cells were obtained. All antibodies secreted by these B cells were observed to bind to all of the PRRSV type 2 isolates tested, but did not bind to PRRSV type 1 isolates, even though serum from the pigs in which the B cells originated from was able to bind all viruses tested (Figure 1). Four out of five isolated antibodies were determined to bind PRRSV GP5 protein. We were not able to specify to which protein the remaining PRRSV-specific antibody (p10c5) bound. However, not all PRRSV proteins were tested for antigen-antibody interactions.
Two monoclonal antibodies were investigated for their ability to neutralize viral infection, the PRRSV GP5 specific antibody (p5c4) and the antibody of unknown PRRSV specificity (p10c5) (Figure 2). Antibody p5c4 neutralized PRRSV ATP, which the pig had been vaccinated against (homologous neutralization). However, it was unable to neutralize PRRSV VR2332 virus, in which the pig had not been exposed (heterologous neutralization). Antibody p10c5 was unable to neutralize either ATP or VR2332 virus, identifying this antibody as PRRSV-specific, but non-neutralizing.

Conclusions:
Isolated antibodies were determined to be homologously neutralizing, suggesting that a polyclonal response, involving multiple PRRSV specific antibodies is necessary for PRRSV neutralization. GP5 was confirmed as a neutralizing epitope of PRRSV. However, other viral proteins are likely involved, but not yet identified. An isolated PRRSV specific antibody was determined to be broadly binding, but only homologously neutralizing, suggesting that broad binding of antibodies against PRRSV precede or are involved in broad neutralization. This means that vaccination aiming to protect from infection may involve exposure to multiple strains of PRRS viruses, as opposed to a single strain of vaccine, in order to induce a broadly protective immunity.

	Sample	ATP (PRRSV-2)	VR2332 (PRRSV-2)	MN 184 (PRRSV-2)	174 (PRRSV-2)	144 (PRRSV-2)	134 (PRRSV-2)	1-26-2 (PRRSV-2)	SDEU (PRRSV-1)	LV (PRRSV-1)
	BNW4 serum	+	+	+	+	+	+	+	+	+
	BNW7 serum	+	+	+	+	+	+	+	+	+
Isolated PRRSV-Specific Abs	BNW4 p14	+	+	+	+	+	+	+	-	-
	BNW7 p1c1	+	+	+	+	+	+	+	-	-
	BNW7 p5c4	+	+	+	+	+	+	+	-	-
	BNW7 p9	+	+	+	+	+	+	+	-	-
	BNW7 p10c5	+	+	+	+	+	+	+	-	-
	Pos ctrl (commerical antibody)	+	+	+	+	+	+	+	+	+
	Neg ctrl (PRRSV naive serum)	-	-	-	-	-	-	-	-	-

Figure 1: Ability of antibodies produced from immortalized B cells to bind a variety of PRRSV type 1 and type 2 isolates.

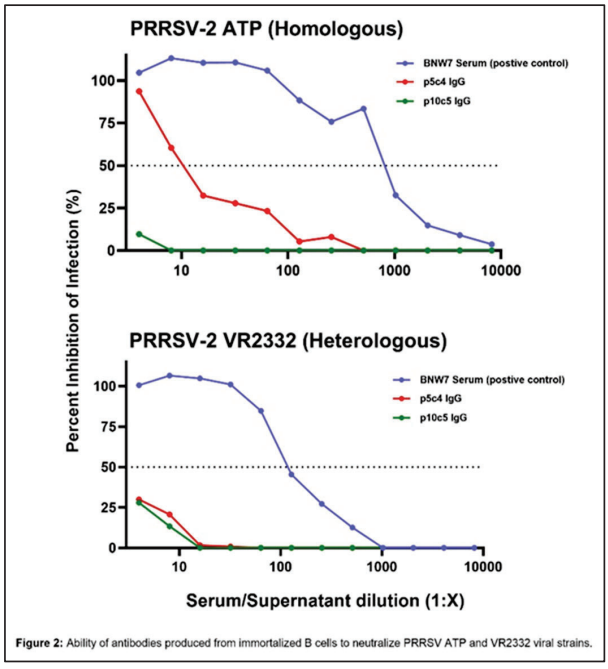

Figure 2: Ability of antibodies produced from immortalized B cells to neutralize PRRSV ATP and VR2332 viral strains.

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Bibliography

1 Lopez OJ, Osorio FA. Role of neutralizing antibodies in PRRSV protective immunity. *Vet Immunol Immunopathol* 2004; **102**: 155–63.
2 Plagemann PGW. Neutralizing antibody formation in swine infected with seven strains of porcine reproductive and respiratory syndrome virus as measured by indirect ELISA with peptides containing the GP5 neutralization epitope. *Viral Immunol* 2006; **19**: 285–93.
3 Robinson SR, Li J, Nelson EA, Murtaugh MP. Broadly neutralizing antibodies against the rapidly evolving porcine reproductive and respiratory syndrome virus. *Virus Res* 2015; **203**: 56–65.
4 Robinson SR, Rahe MC, Gray DK, Martins K V, Murtaugh MP. Porcine reproductive and respiratory syndrome virus neutralizing antibodies provide in vivo cross-protection to PRRSV1 and PRRSV2 viral challenge. *Virus Res* 2018; **248**: 13–23.