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# Safety and attenuation of PRRSV strains genetically modified in the NSP2 region in young pigs

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## Introduction and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly infectious RNA virus endemic in almost all pork producing areas of the world which in many cases causes economic devastation. The implementation of an effective elimination plan for PRRSV will need to incorporate safe and effective vaccines. One approach for the preparation of modified-live virus (MLV) vaccines has been the deletion of regions in the virus that lead to attenuation of virulence. Another new development in PRRSV vaccine design are vaccines that can differentiate infected from vaccinated animals (DIVA).[2] Previously, we reported the use of an infectious cDNA clone to construct two infectious PRRSV strains with deletions in the hypervariable domain of the non-structural protein 2 (nsp2) region.[3] The P129-GFP strain had a normal virus yield and the P129-HA had a 10-fold decrease in virus yield on MARC-145 cells[1] The objective of this study was to evaluate, in young pigs, the safety and attenuation level of two PRRSV strains genetically modified in the nsp2 region. The hypothesis that the nsp2 deleted region will affect nsp2 function and attenuate the virulence properties of the wild-type P129 virus was assessed using clinical, virological and immunological tests.

## Material and Methods

Forty-eight clinically healthy, crossbred PRRSV-naïve pigs were randomized into 4 treatment groups and 2 non-treatment groups. Pigs were blocked by body weight and housed in pens by treatment group, 1 pen per treatment. At 4 weeks of age (Day 0), pigs were administered the assigned treatment, both intranasally (1mL/nostril) and intramuscularly (2 mL/left neck): T01 (n=6)-mock, T02 (n=10)-P129-HA-tag, T03 (n=10)-P129-GFP and T04 (n=10)-P129 wild-type. Two hours after treatment, 2 non-treated pigs each were placed into T02, T03 and T04 pens to serve as contact controls (NT2). Six pigs were housed separately to monitor the health status of the source pigs (NT1).

All pigs were weighed upon arrival, prior to challenge and at necropsy. Following challenge, pigs were monitored for signs of depression, loss of appetite, sneezing, coughing and respiratory distress, and rectal temperatures were recorded on Days -1, 3, 7 and 10. Blood samples, collected from all pigs on Days 0, 3, 7, 10, 14, 21 and 28, were analyzed appropriately for PRRSV isolation (VI), serology (IDEXX ELISA) and/or SN (Serum Neutralization) antibodies. On Days 10 and 28, pigs were euthanized, necropsied, percentage lung lesions scored and tissues collected (lung, inguinal lymph nodes and tonsil) for histopathology and VI.

## Results

- Mock and source control pigs remained healthy with no detection of PRRSV exposure.
- 129P-HA-tag and 129P-GFP pigs remained clinically healthy during the study.
- VI from NT2 pigs was delayed in both 129P-HA-tag and 129P-GFP pigs.
- In general, VI levels were near the lowest detection level in both 129P-HA-tag and 129P-GFP pigs.
- Serology and SN assays indicate a PRRSV immune response in both 129P-HA-tag and 129P-GFP pigs.

## Discussion and Conclusions

The two PRRSV strains genetically modified in the NSP2 region were safe in young pigs with no adverse clinical signs observed following challenge. The deleted region in nsp2 affected nsp2 function and attenuated the virulence properties as seen by level and duration of VI with both MLV viruses eliciting an immune response.

Further testing will be required to determine if the immune response is protective against a virulent challenge as well as the functionality of the nsp2 deletion for a DIVA vaccine.

This study was conducted with the approval of the KSU Animal Care and Use Committee, and Institutional Biosafety Committee approval was obtained under IBC#515 to cover the exposure of whole animals to rDNA and the use of genetically modified PRRSV. National Pork Board provided additional funding.

## References

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