

## The Impact of Attenuated Porcine Reproductive and Respiratory Syndrome (PRRS) Vaccine on the Efficacy of Subunit Classical Swine Fever Vaccine<sup>1</sup>

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### Summary

Commercial pigs have been routinely injected with multiple vaccines that are either administered separately or co-administered at the same time for convenience, and to minimize pig stress. However, viruses, including attenuated and modified live virus (MLV) vaccines, can modulate host immune responses that could potentially impact the efficacy of co-administered vaccines. Here we report the effects of pre- and co-administered Chinese highly pathogenic porcine reproductive and respiratory syndrome (PRRS) virus MLV, JXA1-R, on the efficacy of an emulsion-based classical swine fever virus (CSFV) subunit vaccine, KNB-E2. Immune responses to the CSFV and JXA1-R vaccines were evaluated by testing CSFV-specific and PRRSV-specific sera antibodies and then challenged with CSFV at 4 weeks post KNB-E2 vaccination. Pigs co-administered with JXA1-R vaccine and pigs vaccinated with JXA1-R two weeks before KNB-E2 vaccination had slightly lower CSFV-specific antibodies than pigs vaccinated with KNB-E2 alone at 3 weeks post KNB-E2 vaccination. However, both groups of JXA1-R/KNB-E2 vaccinated pigs were amply protected from CSF clinical symptoms upon challenge. The immunological responses affected by various multiple vaccination combinations in swine would be an interesting aspect for future investigations.

### Introduction

Pigs are routinely immunized with multiple vaccines to prevent or control infectious diseases. To save time, labor, and stress of handling pigs multiple times, vaccine companies supply single-dose, multiple-way vaccines comprised of both live (attenuated or modified) and non-live (inactivated, killed or subunit) vaccines. However, live viral vaccines could modulate the host immune responses and potentially affect the efficacy of co-administered vaccines.

<sup>1</sup> This research is supported by awards from the National Bio and Agro-Defense Facility Transition Fund, the USDA National Institute of Food and Agriculture, Hatch-Multistate project 1021491, USDA ARS Non-Assistance Cooperative Agreements (58-8064-8-011, 58-8064-9-007, 58-3020-9-020, and 59-0208-9-222), and National Pork Board Grant #18-059.

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Classical swine fever (CSF) or hog cholera is a highly contagious swine viral disease that causes severe economic losses to the swine industry worldwide. The etiological agent CSF virus (CSFV), is a positive-sense, single-stranded RNA virus that belongs to the Flaviviridae family under *Pestivirus* genus. In CSF-endemic countries, pigs are routinely vaccinated with CSFV vaccines together with other pig vaccines such as PRRS.

The PRRS continues to be one of the most widespread and economically devastating diseases in the swine industry. The causative agent PRRSV is a relatively small, positive-sense, single-stranded RNA virus with observed high-mutation rate due to the nature of its RNA genome. The highly contagious PRRS virus significantly inhibits host immune responses during the early stage of infection.<sup>4</sup> A PRRS infection also leads to significant thymus atrophy and injuries to tonsil and lymph nodes, and therefore, further weakening the immune response. Moreover, PRRSV infection prior to CSFV vaccination significantly suppresses the CSFV-specific antibody responses, and this immune suppression has been previously reported in PRRS MLV vaccine (Ingelvac).<sup>5</sup>

In the present study, we evaluated the effect of an attenuated Chinese highly pathogenic PRRS vaccine, JXA1-R,<sup>6</sup> on the efficacy of CSFV subunit vaccine, KNB-E2,<sup>7,8</sup> in terms of schedule of vaccination.

## Procedures

### *Cells, Viruses, Vaccines, and Vaccination*

Simian kidney epithelial cells (MARC-145), swine testicle (ST) cells, the Chinese highly pathogenic PRRS modified live vaccine (HP-PRRS MLV), JXA1-R, Classical swine fever virus isolate Alfort (HCV Alfort C-718 28), and the CSFV E2 sub-unit vaccine, KNB-E2 were prepared as we described previously.<sup>6,7,8</sup> All vaccinated pigs were immunized once intramuscularly in the neck area with 2 mL of KNB-E2 and/or JXA1-R. A group of positive control pigs were vaccinated with KNB-E2 only. The non-vaccinated, nonchallenged (-/-); and non-vaccinated, CSFV-challenged (-/+), negative control pig groups were injected intramuscularly with 2 mL of PBS.

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<sup>6</sup> Galliher-Beckley, Amy, Li, X., Bates, J. T., Madera, R., Waters, A., Nietfeld, J., Henningson, J., He, D., Feng, W., Chen, R., and Shi, J. 2015. Pigs immunized with Chinese highly pathogenic PRRS virus modified live vaccine are protected from challenge with North American PRRSV strain NADC-20. *Vaccine*, 33(30), 3518–3525. <https://doi.org/10.1016/j.vaccine.2015.05.058>.

<sup>7</sup> Madera, R., Gong, W., Wang, L., Burakova, Y., Llellish, K., Galliher-Beckley, A., Nietfeld, J., Henningson, J., Jia, K., Li, P., Bai, J., Schlup, J., McVey, S., Tu, C., and Shi, J. 2016. Pigs immunized with a novel E2 subunit vaccine are protected from subgenotype heterologous classical swine fever virus challenge. *BMC Veterinary Research*, 12(1), 1–10. <https://doi.org/10.1186/s12917-016-0823-4>.

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### ***Animals and CSFV Challenge Study***

Conventional Large White-Duroc crossbred weaned specific-pathogen free female piglets (3 weeks of age) were purchased from a commercial vendor. The pigs were fed with standard commercial diet and kept under laboratory biosafety level II (BSL2) conditions at the Large Animal Research Center (LARC) during the vaccination phase; and under laboratory biosafety level III Agriculture (BSL3-Ag) conditions at the Biosecurity Research Institute (BRI) during the CSF challenge phase. Both facilities are at Kansas State University. Animal care and use protocols were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC No. 3892). To test the efficacy of KNB-E2 in pigs vaccinated with JXA1-R, 25 pigs were randomly allotted into five groups: (1) non-vaccinated, non-challenged pigs (-/-); (2) non-vaccinated, CSFV-challenged (-/+ ) pigs; (3) pigs that were vaccinated with JXA1-R and then vaccinated with KNB-E2 after two weeks (*pre JXA1-R + KNB-E2*); (4) pigs that were vaccinated with JXA1-R and KNB-E2 at the same time (*JXA1-R + KNB-E2 co-vax*); and (5) pigs that were single vaccinated with KNB-E2 (*KNB-E2 ctrl*). All KNB-E2 vaccinations were performed on the same day, at two weeks post JXA1-R vaccination of *pre JXA1-R + KNB-E2* pigs. Four weeks after KNB-E2 vaccination, the pigs were challenged with  $1 \times 10^5$  TCID<sub>50</sub> CSFV isolate Alfort (1 mL, intramuscularly). Pigs were monitored daily for clinical signs and rectal temperatures after CSFV challenge. Blood was collected weekly during the vaccination phase and every 3 days during the challenge phase. End of study was at 15 days post challenge (DPC).

### ***Serum Isolation, ELISAs, and CSFV Detection, Virus Neutralizing Titer in Serum, and Statistical Analysis***

Serum was separated from clotted blood and preserved at -20°C until used for assays. Serum isolation, ELISAs, and CSFV detection, virus neutralizing titer in serum, and statistical analysis were performed as we described previously.<sup>4</sup>

## **Results and Discussion**

### ***JXA1-R and KNB-E2 Double Vaccinated Pigs Have Lower CSFV-Specific Antibodies than Single Vaccinated KNB-E2 Pigs***

To determine whether vaccination with JXA1-R could affect the efficacy of KNB-E2 CSF sub-unit vaccine, two groups of pigs were immunized with both JXA1-R and KNB-E2 at different time points (*pre JXA1-R + KNB-E2* and *JXA1-R + KNB-E2 co-vax*) and then challenged with CSFV 4 weeks post KNB-E2 vaccination. No marked differences in the CSFV-specific antibodies were observed between *pre JXA1-R + KNB-E2* and *JXA1-R + KNB-E2 co-vax* pig groups (Figure 1). Interestingly, the CSFV-specific antibodies observed in JXA1-R and KNB-E2 double vaccinated pigs appear to be lower (difference not statistically significant) than single vaccinated *KNB-E2 ctrl* pigs at 4 weeks post KNB-E2 vaccination. The observed difference in CSFV-specific antibodies, although minimal, possibly indicate an effect of JXA1-R vaccination.

### ***JXA1-R and KNB-E2 Double Vaccinated Pigs Were Protected Against CSF***

Upon CSF challenge, all single and double vaccinated pigs did not exhibit CSF clinical symptoms. These single and double vaccinated pigs steadily gained weight throughout the 15-day CSFV challenge period (Figure 2). In contrast, weight loss was observed in the negative (-/+) CSF-challenged control group starting at 6 DPC. Elevated tempera-

tures were also observed in this control group (Figure 3). Although slightly lower CSFV-specific antibodies were observed in double vaccinated pigs during the vaccination phase, the continued body weight gain, and the absence of elevated temperatures in single and double vaccinated pigs during the challenge phase indicate sufficient protection by KNB-E2 vaccination against CSF disease.

To further determine whether the timing of JXA1-R vaccination influenced the induced CSF-specific immune responses by KNB-E2, CSFV quantification was determined by real-time RT-PCR in which positive results were considered for threshold cycle values (Ct) equal to or less than 40. Samples in which fluorescence was undetectable were considered negative. CSF was detected starting at 3 DPC (Figure 4). The *pre JXA1-R + KNB-E2* pigs also tested positive at 3 DPC like the (-/+ ) control pigs but fluorescence detected did not go as high as the (-/+ ) control pigs. However, *pre JXA1-R + KNB-E2* pigs tested CSF negative at 9 DPC and again tested positive at 12 DPC which suggests the persistence of CSF virus throughout the challenge period in this pig group (Figure 4). In contrast, the *JXA1-R + KNB-E2 co-vax* and *KNB-E2 ctrl* pig groups transiently had little or no virus detected at 6 DPC and were quickly cleared afterwards (Figure 4). Of note, CSFV was not detected in the *KNB-E2 ctrl* pig group by 9 DPC. This finding suggests that the observed decrease in body weight gain (Figure 2) and slightly elevated temperature (Figure 3) in *KNB-E2 ctrl* pig group towards the end of the study were due to unrelated non-CSFV health issues.

To determine the amount of virus neutralizing antibody (VNA) titers present in the blood, pig sera were incubated with CSFV for an hour, cultured in ST cells for 72 hours, and virus infection was visualized by immunofluorescence staining. Low levels of anti-CSFV neutralizing titers were detected in all vaccinated groups before challenge with the notably lower VNA titers in the *pre JXA1-R + KNB-E2* pig group. However, by the end of the study (15 DPC) all the vaccinated pigs groups developed very high VNA titers (data not shown). Interestingly, all the (-/+ ) control pigs developed low levels of VNA titers by 15 DPC. However, the VNA titers of the (-/+ ) control pigs were still significantly lower compared with all the vaccinated pigs. Taken together, our data suggest that KNB-E2 remain efficacious in pigs that are pre-vaccinated or co-vaccinated with the PRRS MLV vaccine JXA1-R. However, the subtle differences observed in the double vaccinated pigs could indicate an unaccounted effect of modified live virus vaccination.

Our results show the effects of Chinese highly pathogenic PRRS vaccine, JXA1-R, on the efficacy of CSFV subunit vaccine, KNB-E2, in which the effects were determined by CSFV Alfort challenge. All JXA1-R and KNB-E2 double vaccinated pigs were protected against CSF clinical symptoms. These pigs displayed continued body weight gain, no CSF clinical symptoms, and the absence of high fever after CSFV infection. In contrast to (-/+ ) unvaccinated CSF challenged pigs, all JXA1-R and KNB-E2 double vaccinated pigs exhibited no or low levels of CSF viremia. The *JXA1-R + KNB-E2 co-vax* had lower CSFV E2-specific and neutralizing antibodies but developed very high antibody levels during the challenge phase. The *pre JXA1-R + KNB-E2* pigs had displayed higher PRRS-specific antibodies at 2 weeks post JXA1-R vaccination than *JXA1-R + KNB-E2 co-vax* pigs and lower CSF-specific antibodies at 4 weeks post KNB-E2 vaccination. Although the retained efficacy displayed by the KNB-E2 vaccine

illustrates the minimal impact of JXA1-R pre-vaccination and co-vaccination, notable differences were observed between single and double vaccination.

Taking into consideration that infection with various PRRSV strains could inhibit the replication and efficacy of C-strain CSF MLV<sup>9</sup> and that KNB-E2 vaccinated pigs are protected against CSF challenge even with prior or co-PRRSV vaccination, the use of KNB-E2 CSF vaccine could be a potential alternative to C-strain CSF MLV vaccination. This subunit vaccine could confer protection against CSF in a single dose vaccination at two weeks post vaccination.<sup>4,5</sup> It induces strong immune responses against CSFV without the untoward effects of immune deregulation induced by virus-based vaccines.

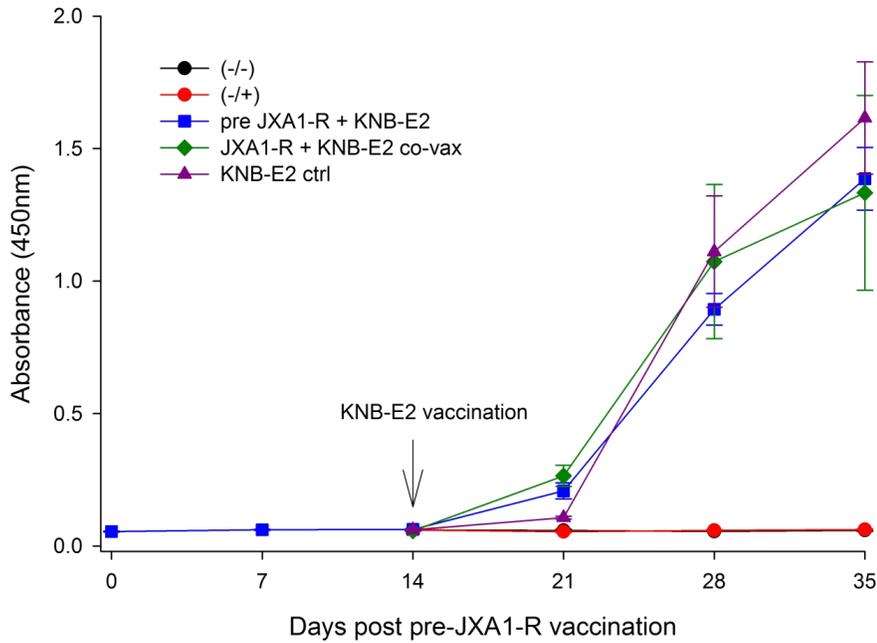
The effects of pre- and co-vaccination of JXA1-R and KNB-E2 were not tested against PRRSV challenge. Results indicate slightly lower PRRS-specific antibodies at 2 weeks post JXA1-R co-vaccination in comparison with JXA1-R pre-vaccination (data not shown). However, the observed S/P values (>0.4) on these pigs show successful sero-conversion and this is an indication of elicited immunity against PRRSV.

We have demonstrated JXA1-R PRRS vaccine to be safe for use in multiple-dose vaccination with little or no effect on the efficacy of KNB-E2 CSF subunit vaccine. The Chinese highly pathogenic PRRS virus MLV, JXA1-R appears to have minimal immune suppression and no effects on subunit KNB-E2 CSF vaccine efficacy. Although no drastic differences were observed between pre-vaccination and co-vaccination of the vaccines, the results, nevertheless, indicate that the effect of multiple vaccinations in pig immunization schedules needs to be further addressed.

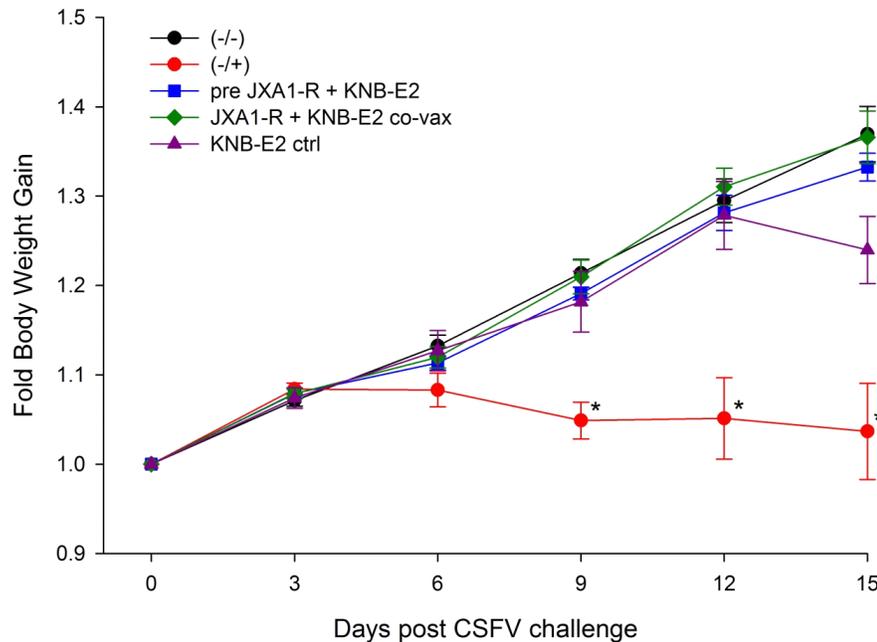
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## Swine Day 2020

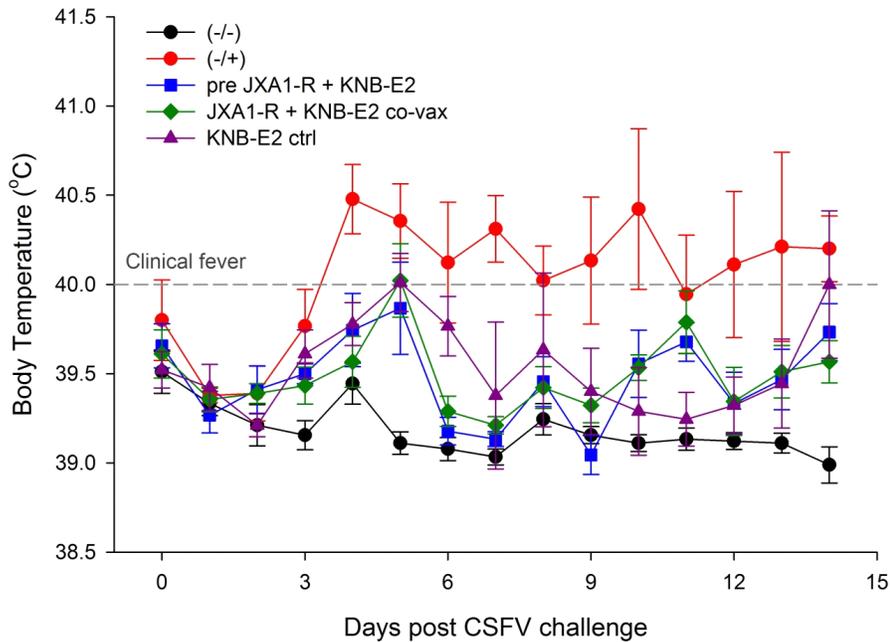


**Figure 1. Anti-CSF antibody responses of JXA1-R and KNB-E2 pre- and co-vaccinated pigs post vaccination. Pigs pre-vaccinated with JXA1-R (pre JXA1-R + KNB-E2) and pigs vaccinated with JXA1-R and KNB-E2 at the same time (JXA1-R + KNB-E2 co-vax) displayed slightly lower anti-E2 CSF antibodies compared with pigs that were vaccinated only with the classical swine fever sub-unit vaccine (*KNB-E2 ctrl*). ELISA plates coated with 62 ng/mL E2 antigen and serum samples diluted 1:1,000. Data are group mean  $\pm$  SEM (n = 5) absorbance determined by E2-specific ELISA.**

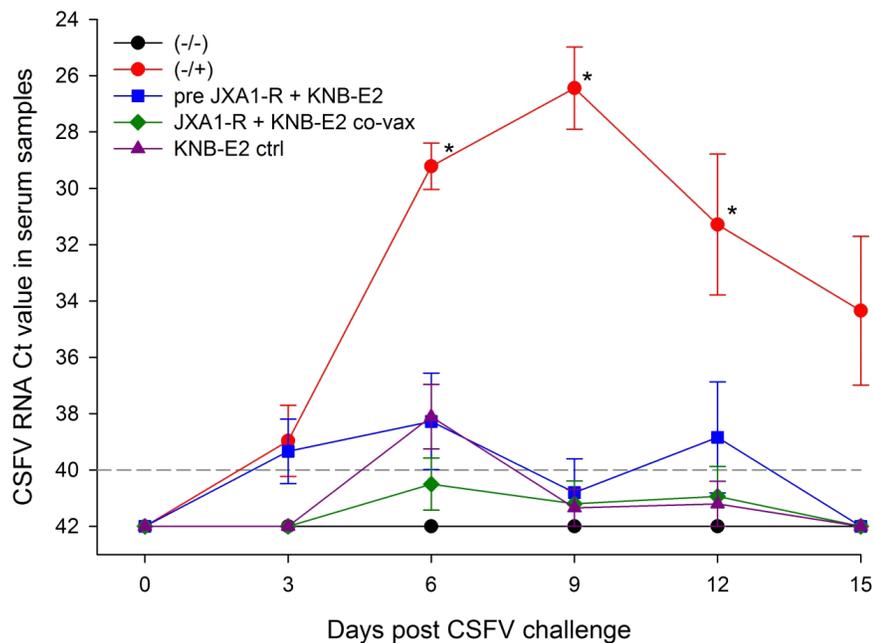


**Figure 2. Body weight gain post CSF challenge of JXA1-R and KNB-E2 pre- and co-vaccinated pigs. All experimental pig groups displayed continued weight gain post CSF challenge. The observed weight loss at 15 DPC in the pigs vaccinated with KNB-E2 only vaccine (*KNB-E2 ctrl*) was not CSF-related. Data are group mean  $\pm$  SEM (n = 5) fold body weight gain calculated by considering 0 DPC pig weight as 1.**

## Swine Day 2020



**Figure 3.** Body temperature of JXA1-R and KNB-E2 pre- and co-vaccinated pigs post CSFV challenge. Pigs pre-vaccinated with JXA1-R (pre JXA1-R + KNB-E2), pigs vaccinated with JXA1-R and KNB-E2 at the same time (JXA1-R + KNB-E2 co-vax) and pigs vaccinated with KNB-E2 only (*KNB-E2 ctrl*) did not develop clinical fever after challenge with CSF virus. Data are group mean  $\pm$  SEM (n = 5) temperature ( $^{\circ}$ Celsius).



**Figure 4.** Viremia of JXA1-R and KNB-E2 pre- and co-vaccinated pigs post CSFV challenge. Pigs pre-vaccinated with JXA1-R (pre JXA1-R + KNB-E2), pigs vaccinated with JXA1-R and KNB-E2 at the same time (JXA1-R + KNB-E2 co-vax) and pigs vaccinated with KNB-E2 only (*KNB-E2 ctrl*) did not develop high levels of CSFV viremia. Samples with real-time RT-PCR threshold cycle (Ct) values equal or less than 40 were considered positive for CSFV. Data are group mean  $\pm$  SEM (n = 5) Ct values.