RESEARCH ABSTRACT

SWINE HEALTH

Title: Development and validation of two anti-CSFV-specific antibody competitive ELISAs with an emphasis on the differentiation of infected from C-strain vaccinated animals - **NPB #18-059**

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Scientific Abstract:

Introduction

Currently, CSF continues to cause severe economic losses to the swine industry worldwide. The conventional Chinese vaccine strain (C-strain) is the most frequently used vaccine for CSF control and prevention, and has showed outstanding efficacy and safety after many years of use. However, no reliable DIVA (differentiate pigs infected with wildtype CSFV from vaccinated animals) diagnostic assay is available for use in conjunction with C-strain vaccination. In this study, we developed and validated two competitive ELISAs (cELISA) with an emphasis on the differentiation of infected from C-strain vaccinated animals.

Method

For generating suitable capture recombinant antigens, E2 protein and E^{rns} protein of C-strain were expressed in insect cells by using Bac-to-Bac® Baculovirus Expression System. For generating suitable competitive monoclonal antibodies (mAbs), purified E2 and E^{rns} of C-strain were used as immunogens to inject Balb/c mice. The generated mAbs were comprehensively assessed by testing insect cell expressed E2 or E^{rns} proteins of CSFVs from genotypes:1.2,1.3,1.4, 2.1a, 2.1b, 2.1c, 2.1g, 2.1h, 2.1i, 2.1j, 2.2, 2.3, 3.1, 3.2, 3.4) using Western blotting, by testing CSFVs (n=111) from genotypes: 1.1, 2.1a, 2.1b, 2.1c, 2.1g, 2.1h, 2.1i, 2.1j, 2.2, 2.3 and testing BVDVs from genotypes: 1 and 2 using indirect fluorescent antibody assay (IFA). cELISAs were established based on the strategy that C-strain specific mAbs will compete with the C-strain vaccine induced antibodies in the pig serum to bind the capture antigens (C-strain E2 or C-strain E^{rns}). The cELISAs were optimized and were further evaluated by testing different categories of pig sera.

Results

i) C-strain E2 and E^{rns} proteins were successfully expressed. The purified native C-strain E2 protein mainly existed as homodimer. The purified native C-strain E^{rns} protein existed as homodimer and monomer; **ii)** Two panels of mAbs were generated. One mAb against E2 protein and one mAb against E^{rns} showed the best differential characteristics; **iii)** Two cELISAs based on these two mAbs were developed. After comprehensive assessment and validation, the HRP-mAb 1504 (against C-strain E^{rns} protein) based cELISA showed high specificity, sensitivity and reproducibility; **iv)** The established HRP-mAb 1504 based cELISA can efficiently differentiate C-strain or C-strain E^{rns} immunized pigs from other CSFVs or other viruses

(PRRSV, PRV, BVDV) infected pigs. It can detect C-strain induced antibodies as early as 7-14 days post vaccination (DPV) with neutralizing titer 1:5 to 1:15. The diagnostic sensitivity and specificity of the cELISA were 100% (95% confidence interval: 91.2 to 100%) and 100% (95% confidence interval: 99.3 to 100%), respectively.

Conclusion

The novel HRP-mAb 1504 based cELISA established in this project is a valuable tool for measuring and differentiating immune responses to C-strain vaccination in pigs. It is applicable as an accompanying DIVA assay to C-strain originated vaccines (live-attenuated or E2 subunit), can be safely manufactured in the United States and will facilitate the implementation of “vaccination to live” strategy for CSF outbreak control.