Design of chimeric antigens of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) through bioinformatics approaches: a rational model for the development of a diagnostic test

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Brazil is one of the largest producers and exporters of swine meat in the world and therefore it is necessary a more stringent control of diseases affecting the swine herd. In this context, the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is the important etiologic agent that causes significant economic losses. There is not official report of PRRSV in Brazil, but there is the possibility of its introduction by the marketing of matrices and semen from endemic countries and by contact with infected animals of border countries. Considering that the introduction of PRRSV in Brazil would have a major impact on the swine industry and on animal health, this research aims to produce a diagnostic test for PRRSV using chimeric antigens constructed with important regions for antibody recognition of both viral types (I and II). For this study, we constructed two databases with complete nucleotide sequences of GP5 and N genes of types 1 and 2 of PRRSV available in GenBank. The databases contemplated 1,768 complete sequences of GP5 and 91 complete sequences of N. The nucleotide sequences were aligned with MUSCLE algorithm and the alignments were reviewed, edited manually and served as the base file for phylogenetic and polymorphism analysis. Phylogenetic hypotheses were calculated by Bayesian Inference using Mr Bayes software version v3.1.2 and Bayesian Markov Chain Monte Carlo method (MCMC) in four runs with 100,000,000 generations to GP5 gene and 1,000,000 generations for the N gene. After viewing the phylogenetic trees, the nucleotide sequences were separated according to the groups formed (viral types 1 and 2) and translated for detailed investigation of polymorphisms using Python scripts. In this analysis, matrices containing the mutations identified in the datasets and their respective frequencies were calculated. With this information and from the alignment of amino acid sequences of each gene were obtained representative consensus sequences of each gene of each viral type using CLC Sequence Viewer version 7.6. After these analyzes, were performed predictions of transmembrane regions of GP5 protein to determine which fragments would be used in the construction of this synthetic gene. Each protein was built separately by joining the fragments of the two viral types with spacers. The genes of GP5 and N proteins were chemically synthesized and cloned into vectors for bacterial expression system. This project is standardizing the steps of expression and purification of recombinant proteins.

Acknowledgements: CAPES, CNPq, FAPEMIG e FUNARBE.