VAC_08 - Integrating Next-Generation Phage Display and bioinformatics approaches for the screening of B-cell epitopes of different antigens using polyclonal sera

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Introduction: The development of vaccines against complex parasites, such as ticks, commonly requires the use of several antigens to obtain protective immune responses. However, the production of multi-antigen vaccines might not be commercially viable. To overcome this limitation, the screening of epitopes and the production of chimeric antigens have been shown as a promising approach for developing feasible vaccines against complex parasites.

Objectives: Here, we aimed to establish a Next-Generation Phage Display (NGPD) approach for identifying B- cell epitopes using polyclonal sera of anti-tick vaccine-protected bovines.

Methodology: Polyclonal sera previously obtained from immunised bovines with a protective cocktail of tick salivary antigens were used as antibody sources. Total IgG was purified and used to screen two phage display libraries separately displaying linear or constrained random peptides. Phages bound to the antibodies were recovered by competitive assay with each antigen, and the peptide-coding region was amplified by PCR and submitted to Next-Generation Sequencing (NGS). The NGS data were applied to a bioinformatics pipeline, as follows: (1) identification of the peptide sequences and their frequencies for each sample and vaccine antigen by gPhage algorithm; (2) assessing peptide enrichment (Z-score>4) by comparative analysis between peptides selected from immune and non-immune sera; (3) the identification of exclusive peptides for each antigen; and (4) the identification of the potential protective epitope in the three-dimensional (3D) structure of each antigen by PepSurf on protein models predicted by RoseTTAFold.

Results: Data analysis from three different antigens showed between 16,627 and 28,552 different linear or constrained peptides selected by the bovine antibodies. After a comparative analysis of the enriched peptides in immune vs non-immune sera, between 1,422 and 3,061 motifs or peptides (~10%) were found to be exclusive from each antigen. Finally, the tracking of these peptides on the surface of the high quality 3D structure model of the respective antigen revealed at least one epitope for each vaccine antigen.

Conclusion: Our study has shown NGPD integrated to bioinformatics approaches that are capable of highlighting potential epitopes of vaccine antigens screened by vaccine-protective polyclonal sera. Financial support: FAPESP 2015/09683-9 and 2022/07400-3, CAPES, CNPq.

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