7TH GLOBAL FORUM ON TB VACCINES | 8-10 October 2024 Rio de Janeiro, Brazil
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Genetic Modification of BCG by CRISPR technology for DIVA Skin Test Compatibility in Bovine Tuberculosis Control

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Introduction: Early tuberculosis diagnosis and vaccination are essential for mitigating its impact on public health and the livestock economy. Existing diagnostic methods face challenges in detecting latent infections, ensuring accuracy, and improving accessibility, particularly in resource-limited settings. The DIVA skin test is a novel veterinary diagnostic tool created to differentiate vaccinated from tuberculosis-infected animals.

Objectives: This study aims to genetically modify the BCG vaccine to enable compatibility with the DIVA skin test for bovine tuberculosis control. Methodology: We applied our previously generated CRISPR/Cas9 system (pKLM-CRISPR) to target five DIVA antigens (mpb70, mpb83, espA, espC, and esxS) from BCG. Transformants underwent PCR screening, followed by genetic characterization through Sanger sequencing. Genetic mutations were evaluated for prediction of peptide interactions with bovine MHC Class II BoLA-DRB3 using NetBoLAIIpan-1.0, to confirm elimination of antigenic sites.

Results: We have obtained four of the five knock outs of the target genes. In mpb70, two clones showed nucleotide insertions, and one had a 250 bp deletion, removing the start codon. For mpb83, the absence of amplicon suggested a deletion beyond 306 bp, also removing the start codon. In espC, a deletion near the 3'-end affected an important MHC-II DRB3 recognition region. In esxS, one clone had a downstream 2156 bp deletion, including neighbouring genes, and a 993 bp inversion, while the other had a 107 bp deletion.

Conclusion: We have developed a time-saving genetic modification approach for vaccine development, capable of producing unmarked recombinant BCG knock-out strains. The next steps involve the concomitant deletion of all these genes and DIVA antigen production. The resulting BCG knock-out will be evaluated in vivo to confirm its suitability for use in combination with specific DIVA skin tests. Our future perspective is to proceed to field trials.

Funding Sources
FAPESP and Fundação Butantan

Conflicts of Interest
None