Title: CHARACTERIZATION AND EVALUATION OF IMMUNOGENICITY OF ENOLASE GENE *Conidiobolus lamprauges*

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Abstract:

Conidiobolomycosis is a disease associated to fungus *Conidiobolus lamprauges* (*C. lamprauges*), characterized by rhinitis or chronic granulomatous rhinopharyngitis and affects animals and humans. Enolase, an important glycolytic enzyme with increased expression at 37° C in *C. lamprauges*, has different functions in other organisms, among them, adhesion and invasion of hosts. Identification of immunoreactive proteins contributes to the development of serological techniques, as well as understanding of the pathogenesis of the disease. Thus, it can assist in a more efficient treatment and, consequently, reducing the death of the animals. There are no vaccines or treatments for this pathology both for humans and animals and identification of immunoreactive proteins could allow new opportunities for the prevention or treatment thereof. The objectives of this study were to characterize the enolase gene (eno) *C. lamprauges* and evaluate their immunogenic capacity to naturally infected sheep sera. Gene amplification eno from *C. lamprauges (*FIOCRUZ INCQS 40316), based on oligonucleotides sequence of the gene *C. lamprauges* resulted in 1305 pb fragment. The deduced amino acid sequence was 434 residues with a predicted molecular weight of 47,2 KDa. In the following analysis, we identified the signature of enolase (LLLKVNQIGTVSES) corresponding to positions 342-345. In the analysis "in silico" probable regions were detected for epitopes to T and B lymphocytes. The sequence was cloned into the plasmid *pFN6K (HQ) Flexi® Vector* (Promega®) and the protein expressed in *Escherichia coli BL21 (DE3) pLysS* cells with IPTG induction. In SDS-PAGE, the recombinant ENO was detected with estimated molecular weight of 47kDa. Recombinant IgG anti-enolase was detected in all animals with conidiobolomycosis by Western blot demonstrating immunogenicity. In healthy sheep serum, used as a negative control and serum from sheep with pythiosis used to evaluate cross-reactivity, bands were not detected. Therefore, this study demonstrated that recombinant ENO of *C. lamprauges*, is immunogenic against sheep naturally infected sera with conidiobolomycosis. The detection of specific anti-enolase antibodies in animals show that the protein is a promising candidate antigen for the development of effective vaccine against the disease or diganostic test

Keywords: enolase, Western blot, ELISA, Conidiobolus lamprauges, sheep

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