

TITLE: IMMUNOGENICITY OF *Pasteurella multocida* RECOMBINANT PROTEINS OBTAINED FROM TRANSCRIPTS EXPRESSED DURING IN VIVO INFECTION

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ABSTRACT: *Pasteurella multocida* (*P. multocida*) is a Gram-negative coccobacillus, classified in five serotypes (A, B, D, E and F), causing a variety of diseases in several hosts and in swine related to respiratory problems. Based on data obtained during the infection in swine by *P. multocida*, it was possible to select transcripts that induce immune response, allowing the production of recombinant protein vaccines. This study aimed to characterize protective effects of three recombinant proteins Adhesin-YadA proteins, Periplasmic protein probably involved in high-affinity Fe²⁺ transport and Trap-T family transporter of *P. multocida* by the experimental immunization in mice. The genes were amplified by PCR and cloned into the vector pNF6-K, sequenced and then transformed into *E. coli* BL21 (DE3). Protein expression was induced by the addition of IPTG and purified in histidine tail system, and then analyzed on SDS-PAGE and Western blotting using serum from infected swine. Recombinant proteins were conjugated to the adjuvant (aluminum hydroxide) and inoculated to separate mice in groups: Adhesin (GA); Fetrans (GF); Trap (GT). The animals of the *P. multocida* Group (Gpm) and Control Group (CG) were not immunized. Two doses of the conjugate were administered at 21 day intervals and then the animals (GA, GF, GT and Gpm) were challenged with suspension of *P. multocida* via the intranasal route. After challenge, 60% of the Gpm animals died from 20 to 40 hours post challenge. In the GA, GF, GT and GC groups there was 100% survival. The tissue alterations in Gpm were accentuated pulmonary congestion with alteration of pleural surface coloration and edema and accentuated perivascular suppurative inflammatory infiltrate. In animals immunized with recombinant proteins, consolidation in the cranial portion of the pulmonary lobes was observed only in GA. Microscopically the GA, GF and GT groups presented slight alterations mainly hyperplasia of BALF follicles. Based on our results, we conclude the immunological potential of the Adhesin-YadA proteins, Periplasmic protein probably involved in high affinity Fe²⁺ transport and Trap-T family transporter, with 100% protective index of the vaccinated animals, besides promoting tissue immune response pulmonary hyperplasia of BALF follicles.

Keywords: Pasteurethosis, immunogenicity, recombinant proteins, vaccines.

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