Production of a recombinant *Cryptosporidium parvum* 12kDa protein in *Escherichia coli* and development of specific antibodies

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Abstract

Introduction: Parasite recombinant proteins are gaining wide-spread attention due to its diagnostic application. This work aims at the evaluation of a novel fusion system for the production of a 12 kDa surface adherence protein from *Cryptosporidium parvum*, which has been reported as a potential target for cryptosporidiosis prevention and therapy [Yao et al, 2006]. It is also intended to obtain specific antibodies and to study its ability to recognize the surface of sporozoites and oocysts.

Methods: Specific primers were designed to amplify and sub-clone part of the gene that encodes for *Cryptosporidium parvum 12kDa* protein lacking its transmembrane domain. This truncated protein was expressed in *E. coli* using a novel fusion system that significantly increases protein production yields. After its purification by affinity chromatography on Ni-NTA columns, the protein was dialysed against phosphate buffer pH 8.0 and later injected in mice. The immune response obtained in mice was then evaluated by ELISA and immunofluorescence assays.

Results: The expression levels of fused protein in *E. coli* increased about three folds when compared to the respective non-fused protein levels Results from ELISA and immunofluorescence assays confirmed the potential diagnostic interest of this recombinant antigen.

Conclusions: Results from this work may provide an important advance on diagnostic and therapeutic field on cryptosporidiosis.