The recombinant cathepsin B3 from *Fasciola hepatica* as a potential diagnostic factor.*

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Introduction and Aim. *Fasciolosis* is an important zoonotic disease that is responsible for a significant loss in the agriculture industry and animal productivity, primarily through infection of sheep and cattle. Losses in animal productivity due to liver fluke have been estimated at over US$3.2 billion per annum (1). Cathepsin L and B are the most highly expressed cysteine proteases secreted in various development stages of *Fasciola* (2). FhCB3 has been detected as one of the major component of somatic extract of newly excysted juvenile (NEJ) (3).
The aim of this study was to examine the usefulness of recombinant cathepsin B3 from *Fasciola hepatica*, expressed in prokaryotic and eukaryotic system in diagnosis of fasciolosis.

Materials and Methods. The FhCB3 (GenBank: EU090822.1) cDNA sequence was cloned into pET28a(+) and pPICZαA. Additionally, sequence without potential N-glycosylation sites was cloned into pPICZαA. Recombinant FhCB3 was expressed in *E. coli* and *P. pastoris* and purified by HIS-Select® HF Nickel Affinity Gel. Obtained proteins were used as coating antigens in ELISA with rat and sheep serum which were infected with *F. hepatica*, and for panning of mouse and rat phage display libraries (constructed in our laboratory) in order to obtain specific monoclonal antibodies.

Results. The FhCB3 containing a propeptide region and tagged with hexahistidine was expressed in *P. pastoris* and *E. coli*. Western blot and SDS- PAGE analyses of the purified protein revealed a band at about 38 kDa and a smear ranging from 55-75 kDa for protein obtained in yeast and a band at ~36 kDa for protein produced in bacteria. The higher protein band in yeast expression correspond to glycosylated protein fraction which was confirmed in glycoprotein staining assay. The purified proteins were stable after dialysis to PBS and storing in -80 °C. Specific antibodies against FhCB3 begin to appear at 2 week post infection in rats. A few highly reactive monoclonal antibodies obtained after panning of both phage display libraries were selected for further analyses.

Conclusion. FhCB3 as one of the main secreted antigens by NEJ *F. hepatica* proved to be a promising diagnostic factor due to early appearance of specific antibodies in animals infected with *F. hepatica*. Additionally, obtained monoclonal antibodies will be used for detecting the CB3 antigen in sera or faeces of infected animals in future experiments.

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