ASPARAGINYL ENDOPEPTIDASE FROM THE CARCINOGENIC LIVER FLUKE, *OPISTHORCHIS VIVERRINI*, AND ITS POTENTIAL FOR SERODIAGNOSIS

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**Objectives:** To isolate and characterize an asparaginyl endopeptidase from the carcinogenic liver fluke, *Opisthorchis viverrini*, and evaluate its expression profile, biochemical activity and potential as an immunodiagnostic antigen.

**Methods:** The full length mRNA encoding an asparaginyl endopeptidase (family C13), *Ov-aep-1*, was isolated by immunoscreening a cDNA bacteriophage library of adult *O. viverrini* using sera from patients infected with *O. viverrini*. Investigation of *Ov-aep-1* transcripts in developmental stages of the parasite, and phylogenetic analysis, immuno-histochemical localization and recombinant protein expression and enzymology were employed to characterize the *Ov-AEP-1* enzyme. Immunoblotting was used to assess the potential of this antigen for immunodiagnosis of human opisthorchiasis.

**Results:** *Ov-AEP-1* is characteristic of the C13 cysteine protease family. *Ov-aep-1* transcripts were detected in adult and juvenile worms, eggs and metacercariae. Phylogenetic analysis indicated that *Ov*-AEP-1 was closely related to homologous proteins in other trematodes. Recombinant *Ov*-AEP-1 was expressed in bacteria in inclusion bodies and refolded to a soluble form. Excretory-secretory (ES) products derived from adult *O. viverrini* and refolded recombinant *Ov*-AEP-1 both displayed catalytic activity against the legumain-specific peptide, Ala-Ala-Asn-aminomethylcoumarin. Rabbit antiserum raised to recombinant *Ov*-AEP-1 identified the native AEP-1 protease in both somatic extract and ES products of adult worms. Anti-*Ov*-AEP-1 IgG immunolocalized the anatomical site of expression to the gut of the fluke, implying a physiological role in digestion of food or activation of other digestive enzymes. Recombinant *Ov*-AEP-1 was recognized by serum antibodies from patients with opisthorchiasis but not other helminth infections, with a sensitivity and specificity of 85% and 100% respectively. The positive and negative predictive values are 100% and 67% respectively.

**Conclusions:** The liver fluke, *Opisthorchis viverrini*, has a gut-localized asparaginyl endopeptidase. Refolded recombinant *Ov*-AEP-1 is catalytically active and has potential for immunodiagnosis of human opisthorchiasis.