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OPISTHORCHIS FELINEUS ANTIGENS AND IMMUNODIAGNOSTIC

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Many important pathogens causing human zoonotic disease are transmissible to human beings from animals by eating raw or partially cooked meat or fish [1, 2]. These include a number of fish-borne zoonotic liver flukes, with currently more than 45 million people estimated to be infected. Of these fish-borne trematodes, Clonorchis sinensis, Opisthorchis felineus and Opisthorchis viverrini (family Opisthorchiidae, subfamily Opisthorchiinae) are most important. Opisthorchis felineus is a parasite predominantly of carnivores throughout much of its range and also causing human disease. Opisthorchiasis due to O. felineus has a population of 12.5 million at risk, at least in Kazakhstan, the Russian Federation, Siberia and the Ukraine. An estimated 1.6 million people are infected [1].

In the past years a strong link has been established between long-time infection and the development of cholangiocarcinoma and it is therefore recommendable to have a fast, robust routine diagnostic assay available which could be performed at low cost at local hospitals to prevent asymptomatic chronic infections [3, 4]. Although it is a rare cancer in the world, high prevalence is observed in the endemic region of Opisthorchis viverrini, for example in the northeast of Thailand where there is the highest prevalence of cholangiocarcinoma in the world [5, 6].

At present, microscopic examination of parasite eggs in the faeces of infected individuals is still a standard method for diagnosis of opisthorchiasis. Several attempts have been made to develop a more effective diagnostic assay including recent PCR approaches to detect the parasite’s DNA in stool samples and fish [7, 8]. Attempts to develop differential analysis were taken of opisthorchiid trematodes of the species Opisthorchis felineus and Metorchis bilis using polymerase chain reaction [9].

Native protein from O. felineus has been used to detect specific antibody in serum but is not suitable for routine diagnosis due to the required amounts and low specificity. The techniques of indirect haemagglutination, intradermal test and ELISA were developed and the crude somatic extract of adult worms was employed [10]. Comparing among these methods, ELISA was found to be the best and the results seemed to correlate satisfactorily with intensity of infection. However, there was no information on the specificity of the test. The O. felineus protein antigens prepared from adult worms, ES and eggs were characterized and found that molecular weight of 105, 74, 70 and 64kDa from adult and egg antigens, respectively, may have potential for immunodiagnosis [11, 12, 13].

In our work we carried out experiments to obtain excretory-secretory antigens of the O. felineus pathogen. A sexually mature marita obtained by infection of golden hamsters via opisthorchis metacercariae isolated from fish of the carp family [14]. To obtain excretory-secretory antigen cultured viable O. felineus marita 14-16 hours in a simulated RPMI-1640 medium. Electrophoretic analysis demonstrated a composition of antigen 21 major protein with a molecular weight of 283, 262, 248, 237, 220, 212, 178, 119, 73, 67, 63, 55, 53, 51, 47, 46, 38, 30, 28, 27 and 25 kDa. A more detailed analysis was carried out by mass spectrometry. The results showed the presence of antigen in the composition of more than 350 proteins of different nature. The dominant majority are excretory proteins having diagnostic value, including paramyosin, glutathione transferase and cathepsin F. Further, our work is in the possibility of produce the recombinant analogues of the above proteins and their use in diagnostic opisthorchiasis.

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