

Detection and characterization of *Dirofilaria repens* immunogenic antigens

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Dirofilaria repens is a causative agent of subcutanoeus dirofilariosis. As a result of climate change and human activities this nematode spread throughout the central and eastern parts of Europe. Although dogs are the main hosts, *D. repens* shows zoonotic potential and can also accidentally infect humans. Nowadays the only available detection method is Knott's test and/or PCR. However, both methods have some limitations caused by microfilarial periodicity. Therefore, there is a great need to explore more the parasite biology that will contribute and enable development of new diagnostic method.

In the present study, our aim was to investigate the highly immunogenic antigens from adult and microfilariae stage of *D. repens* and produce the highly reactive antigens in recombinant form in bacterial and yeast expression systems. We identified stage specific proteins that were recognized by sera of *D. repens*-infected dogs using two-dimensional gel electrophoresis (2DE), 2D-immunoblotting, and LC-MS/MS mass spectrometry. Selected, immunoreactive proteins were evaluated with bioinformatic tools to estimate their potential as a diagnostic antigens. We analyzed homology of selected antigens with protein sequences from other filarial worms like *Dirofilaria immitis*, *Onchocerca volvulus*, *Loa loa* and nematode parasites occurring in Poland e.g. *Toxocara canis*. The antigens of interest were amplified by RACE-PCR method, cloned and sequenced by Sanger's method. Newly learnt *D. repens* proteins were produced in bacterial and/or yeast expression system and purified by affinity chromatography. Purified recombinant antigens were reactive with sera from infected dogs which we tested by Western Blot technique. Additionally we analyzed gene expression level of one target protein in both, adult and microfilariae stage and identified 116 times higher expression in adult *D. repens* parasite, which may indicate the important role of this protein in adult stage of parasite. Further studies are required to test specificity and sensitivity of target proteins to consider them as a potential diagnostic antigens.

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