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Study of serum metabolom in canine babesiosis by mass spectrometry

Ivana Rubić[a], Anita Horvatić[a], Richard Burchmore[b], Clement Regnault[b], Suzanne McGill[b], Ana Monteiro[b], Jelena Gotić[a], Renata Barić Rafaj[c], Vladimir Mrljak[a]*

[A] Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia.
[B] Institute of Infection, Immunity and Inflammation and Glasgow Polyomics, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK

[C] Department for Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia.

INTRODUCTION

Canine babesiosis is an important worldwide tick-borne disease caused by the intra-erythrocyte protozoal parasites *Babesia canis* or *Babesia gibsoni* (Beck et al., 2009). Although the disease process primarily affects erythrocytes, it may also have multisystemic consequences (Barić Rafaj et al., 2013). The main complications are the development of an excessive inflammatory response called "systemic inflammatory response syndrome" or SIRS (Bone et al., 1992) and also a "multiple organ dysfunction syndrome" or MODS (Jacobson and Clark, 1994). Specific metabolites are being discovered as biomarkers to improve disease diagnosis, prognosis, and treatment outcomes. The emergence of innovative, post-genomic technologies, has led to the development of strategies aimed at identifying specific and sensitive biomarkers among thousands of molecules present in biological fluids and tissues (Moore et al., 2007). Nowadays, according to its great potential for biomarkers evaluation, metabolomics is one of the most frequently applied approaches in the field of systems biology (Robinson et al., 2014). Blood and urine contains a multitude of unstudied and unknown biomarkers that may reflect physiological and pathological states of tissues and organs. Particularly low-molecular-weight region of metabolome from blood samples is an important source of diagnostic biomarkers. The goal was to examine the difference of serum metabolome between dogs naturally infected with *Babesia canis* and healthy dogs using liquid chromatography coupled to mass spectrometry (LC-MS).

METHODS

Serum was collected from 12 dogs naturally infected with *Babesia canis* and 12 healthy dogs. Briefly, 25 µL serum aliquots were prepared, and 1000 µL of 1:3:1 chloroform:methanol:water was added to precipitate the proteins. The samples were allowed to cool on ice for 30 minutes, vortexed at 4˚C for 5 minutes, and then centrifuge for 3 minutes at 13.000 g at 4˚C. The supernatant (200 µL) was transferred to a screw-top vial and stored at -80˚C until liquid chromatography-mass spectrometry (LC-MS) analysis. Samples were analysed on an Orbitrap Q-EXACTIVE mass spectrometer (Thermo Fisher Scientific) operating in alternating positive and negative modes with mass resolution 70.000 at m/z range 70 – 1050. Analyses were performed using Polyomics integrated Metabolomics Pipeline (PiMP) program specifically designed for metabolomics.

RESULTS

The metabolomics analysis resulted in the annotation of 1802 peaks, 158 of which showed statistically significant differences ($p < 0.05$) between dogs with *Babesia canis* infection and healthy controls. The peaks represent metabolites in positive and negative modes. 22 identified metabolites were significantly changed. The most significant metabolites were Inosine (peak 290, 1280), Hypoxanthine (peak 70, 1241), Choline phosphate (peak 352), L-Kynurenine (peak 199), and L-Cystine (peak 407). Biological functions of differently abundant metabolites indicate the involvement of various pathways in canine babesiosis including aminobenzoate degradation, benzoate degradation, bile secretion, calcium signalling pathway, D-glutamine and D-glutamate metabolism, dioxin degradation, phenylalanine metabolism, and purine metabolism.

CONCLUSIONS

The study confirmed that host pathogen interactions (Dog – *Babesia canis*) can be studied by metabolomics to assess chemical changes in the host, respectively that the differences in serum metabolome between dogs with *B. canis* infection and healthy dogs can be detected with LC-MS method. The non-targeted LC-MS metabolomic's approach profiled the metabolic change in serum from *Babesia canis*-infected dogs.

Key words: dog, babesiosis, metabolomics, mass spectrometry

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