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MINI-REVIEW

Minireview: Applications of NMR-based metabolomics for the detection and characterisation of toxoplasmosis in felids

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Abstract

Toxoplasmosis is an infection caused by the intercellular protozoan parasite *Toxoplasma gondii*. The parasite has the three-stage life cycle: oocysts, tachyzoites, and bradyzoites. Felids are the only known hosts for the sexual reproduction of *T. gondii* and, therefore, play a crucial role in the transmission of toxoplasmosis. A single cat could spread the parasite to many hosts. Due to the intercellular nature of the parasite, *T. gondii* strongly depends on a host's metabolism in order to leverage carbon and nutrient sources. Therefore, the parasite could be detected in body fluids *via* observation and analysis of metabolic alterations. A range of analytical techniques such as nuclear magnetic resonance (NMR), mass spectrometry coupled with liquid chromatography, and Raman spectroscopy could be applied for the analysis of body fluids of infected animals. However, NMR consists of highly specific analytical techniques due to high reproducibility, availability of a variety of databases, and the ability to obtain the structures of unknown compounds. We present the current extent of NMR-based metabolomics on felid toxoplasmosis and suggest future considerations.

KEYWORDS
cat, felid, metabolomics, nuclear magnetic resonance, one health

1 | INTRODUCTION

Toxoplasmosis is an infection caused by an obligate protozoan intercellular parasite *Toxoplasma gondii*, which could infect almost all warm-blooded animals.[1] *Toxoplasma gondii* has several stages of a life cycle: oocysts, rapidly dividing tachyzoite, and slowly growing bradyzoite.[2] These stages could affect the prognoses of toxoplasmosis such as the tachyzoite form leads to an acute infection, whereas the bradyzoite form might be associated with the long-term chronic disease.[2] Moreover, the life cycle of *T. gondii* consists of both sexual and asexual phases. Sexual reproduction involves the formation of oocysts, whereas the asexual phase results in the formation of tachyzoites and, subsequently, bradyzoites.[3]

The parasite has also two host life cycle, where felids are definitive hosts, and non-felids, for instance, dogs and humans, could be intermediate hosts.[4] However, the parasite may also undergo asexual reproduction among the family of *Felidae* that, in such a case, will act as intermediate hosts. *Toxoplasma gondii* could be found in faeces of infected cats, in environments that were contaminated by infected faeces, and in the meat of other infected animals such as sheep, pigs, and cattle.

In cats, *T. gondii* infects and reproduces inside the gut epithelium cells followed by the excretion of oocysts through defecation.[5] Due to the lack of delta-6-desaturase activity in their intestines, felids are only known hosts for the sexual reproduction of the parasite and, therefore, play a crucial role in the transmission of the infection.[1] The absence of delta-6-desaturase activity leads to the excess of linoleic acid, which is required for the sexual development of *T. gondii*. For instance, the sexual development of the parasite was observed in mice, diets of which were inhibited with murine delta-6-desaturase and supplemented with linoleic acid.[6] *Toxoplasma gondii* infectious in cats continues to be a

veterinary and public health concern. Cats are the most important animals in the epidemiology of toxoplasmosis. They may excrete millions of oocysts.[6] A single cat could spread the infection to many hosts.[6] However, questions such as the frequency of oocysts secretion and immunity of oocysts recreation are yet to be answered.[6] Cats of any breed, age, or sex could die of toxoplasmosis.[6] In non-felids, digestion of oocytes could result in the formation of tachyzoites followed by intercellular tissue cysts that could remain in a body in a form of bradyzoite for the whole life of a host.[5] These infected tissues may be consumed by other animals. As a consequence, *T. gondii* could affect the central nervous system, different organs, and potentially lead to a progression of liver diseases.[3]

Clinical toxoplasmosis could be more severe in kittens that frequently develop pneumonia, hepatitis, and encephalitis. Toxoplasmosis could be fatal for cats with severe respiratory and neurological signs.[4]

This minireview will focus on the metabolites associated with the parasite and techniques used to identify toxoplasmosis.

2 | METABOLITES ASSOCIATED WITH *T. GONDII*

The parasite relies on a host cellular metabolism in order to obtain the biosynthetic "building blocks" and energy that is required for their replication.[2] This leads to constant competition between the parasite and its host for both carbon and nutrients sources. For instance, glucose and glutamine are the major carbon sources for *T. gondii*.[7] Due to the intercellular nature of the infection, the parasite could be detected in body fluids. Therefore, infected cells could display different metabolic phenotypes.[3] Such metabolic alterations and dysregulations could provide an insight into the presence of toxoplasmosis.

A number of metabolites that were altered by the parasite have been identified elsewhere.[3] For instance, a reduced level of taurocholic acid, which is involved in bile acid biosynthesis, hupotaurine, and taurine, which is the free amino acid responsible for antioxidant and anti-inflammatory activity, were detected during the acute infection.[3] Such a reduction in taurine might be buffered by increased levels in glutathionylspermidine, trypanothione, and trypanothione disulfide metabolites that are involved in glutathione metabolism during chronic and acute infections.[3] Furthermore, 20% of the glucose catabolised by tachyzoites is converted to lactate even under aerobic conditions. This leads to the production of significant amounts of acetate.[8]

Toxoplasmosis also leads to significant changes in levels of metabolites that are involved in amino acid metabolism. As many unicellular pathogens, *T. gondii* requires exogenous tryptophan, arginine, and tyrosine for the growth.[7] It has been observed elsewhere that both 3-methoxy-4-hydroxyphenylacetaldehyde and L-thyroxine required for tyrosine metabolism were downregulated in chronic and acute-infected mice.[3]

Furthermore, during acute infections, indole-3-ethanol, nopaline, and gamma-glutamyl-gamma-aminobutyraldehyde that are involved in tryptophan, proline, and arginine metabolisms, respectively, were upregulated.[2] Such dysregulation in these amino acids could be due to the auxotrophic nature of the parasite for tryptophan and arginine.[3]

Arachidonic acid may be one of the most important metabolic pathways between acute and chronic toxoplasmosis.[3] Metabolism of arachidonic acid has been reported to be downregulated during acute infection, whereas it was upregulated during chronic infection. Arachidonic acid is the main polyunsaturated fatty acid that is present in phospholipids of cell membranes and could, subsequently, be released and metabolised to a number of eicosanoids due to the response of an inflammatory stimulus.[3] This also includes inflammatory leukotrienes and prostaglandins. Arachidonic acid and its inflammatory metabolites could mediate the regulation of key processes in cells such as survival, chemotaxis, angiogenesis, mitogenesis, migration, and apoptosis.

As a major nutrient for the parasite, purines are also required for nucleic acid synthesis. *Toxoplasma gondii* could import purines in different forms through different transporters.[7] Moreover, due to the upregulation of glutathione metabolism, the liver could buffer oxidative stress that might be associated with the infection. However, the parasite involved mechanisms for exploiting various amino acids and lipids in order to support its proliferation.[7] Other metabolic pathways and altered metabolites have been identified in the liver of infected mice such as primary bile acid biosynthesis, steroid hormone biosynthesis, bile secretion, and biosynthesis of unsaturated fatty acids.[2,3] In addition, the presence of *T. gondii* could lead to alterations in transcripts associated with cellular metabolism, inflammation-mediated chemokine, activation of T cells, and cytokine signalling pathways.[9]

Furthermore, tachyzoites require a large number of lipids. Infected cells could exhibit an excessive abundance of lipid droplets that could be further increased by exogenous oleic acid.[7] *Toxoplasma gondii* then scavenges neutral lipids and oleic acid from host lipid droplets followed by the storage of excess lipids in deposits as triacylglycerides in its cytosol. It has been shown that disruption of such storage pathways is detrimental to both tachyzoites and bradyzoites in vitro.[7]

Other metabolites could be associated with the TCA cycle, glycolysis, and oxidative phosphorylation metabolism. The alteration of metabolism of phagocytic cells such as dendritic cells by the parasite has been studied elsewhere by mass spectrometry coupled with liquid or gas chromatography (LC/MS or GC/MS).[9] Such lactate dehydrogenase activity and glucose uptake were upregulated in *T. gondii* infected bone marrow derived dendritic cell (BMDC) cultures.[9] Upregulation of arginine degradation along with increased arginase-1 activity, ornithine, and proline production was observed.[9] Furthermore, metabolites associated with energy metabolism including glycolysis, the TCA cycle, and oxidative phosphorylation were determined as major discriminants between control uninfected and *T. gondii* infected cells.[9] It has been reported elsewhere that *T. gondii* infected BMDC cells showed an increase in glycolysis intermediates such as fructose 6-phosphate, glucose 6-phosphates, 2-phosphoglycerate, lactate, and pyruvate after 24 hours.[9] In addition, *T. gondii* led to an increase in the expression of arginase and production of L-proline, L-ornithine, L-glutamate, and L-1-pyrroline-3-hydroxy-5-carboxylate.[9]

It has been discovered elsewhere that the parasite has its own unique enzyme called sedoheptulose bisphosphatase, which is not

present in mammalian host cells.[10] It has been concluded that the sedoheptulose bisphosphatase enzyme could drive carbon into the non-oxidative pentose phosphate pathway, where it might be converted into ribose.[10] This leads to an additional pathway for ribose synthesis. Moreover, the nucleotide metabolism of a host was transcriptionally activated during the infection. Also, denosine kinase and hypoxanthine phosphoribosyltransferase enzymes have been found highly expressed in host cells.[10]

In addition, different amino acids have been obtained by MS elsewhere.[7] These amino acids were valine, alanine, glutamine, aspartate isoleucine, leucine, serine, proline, tyrosine, threonine, and methionine.[7] Phenylalaine has been observed by Raman spectroscopy.[7] Therefore, toxoplasmosis could be detected *via* the analysis of metabolites. In order to determine such dysregulation in metabolites, different tests and analytical techniques could be used.

3 | NMR POTENTIAL FOR THE DETECTION OF TOXOPLASMOSIS

The most common test for toxoplasmosis includes the Sabin-Feldman dye test, enzyme immunoassay for detection of IgG and IgM antibodies, immunosorbent agglutination assay for IgG, IgA, and IgM avidity measurements, immunoblotting, real-time PCR, and histological examination of tissues.[5] However, such methods might be time consuming and involve additional preparation of samples. Therefore, several analytical techniques could be applied in order to test different metabolites present in the fluids of infected animals.

Metabolomic research relies on analytical techniques such as NMR, LC/MS, GC/MS, and mass spectrometry along with capillary electrophoresis (CE/MS).[3] The MS analysis remains a standard and commonly used analytical technique for detection of metabolites and related amino acids.[11,12] Even though LC/MS has been widely used due to reproducibility, high sensitivity, and peak resolution, it requires an additional sample preparation.[3] However, NMR is able to analyse compounds that are required for derivatisation or ionisation.[13]

NMR analysis could be performed in vivo, provide results in highly reproducible data, and provide structures of unknown compounds. NMR could also trace different metabolic pathways and fluxes by using isotope labels. Other advantages of NMR over MS are the non-destruction of samples, availability of databases, as well as a relatively high throughout.[12] NMR analysis allows observation and quantification of compounds present in tissues, biological fluids, and cell extracts without an additional sample elaboration.[13]

NMR-based metabolomics plays an important role in studies of complex biological molecules, their metabolic pathways, and interactions.[13] Applications of NMR-metabolomics are based on the diagnosis of the disease, analysis of biochemical pathways, and monitoring effects of medical treatments. Therefore, NMR-based metabolomic analysis especially in conjunction with MS would have great potential in the detection of toxoplasmosis through specific metabolites.

4 | KNOWLEDGE GAPS

There are a number of limitations associated with the detection of toxoplasmosis *via* metabolic dysregulation. One of such limitations is that the full effect of the parasite on the biochemical composition of the host's body fluids remains unknown.[3] Although the intensive research of the effect of *T. gondii* on a host has been carried out, there are still knowledge gaps. For instance, histidine, cysteine, and lysine might be considered essential for the parasite; however, it has not been verified yet.[7] Another limitation is associated with metabolites produced within different stages of the parasite's life cycle. For example, tachyzoite forms of *T. gondii* might rely on the uptake of spermine and ornithine in order to reverse synthesise polyamines. However, the metabolic roles of these amino acids in the parasite are still unknown.[7] Moreover, it is not well understood how different stages of the parasite interact with the host cell and which metabolic factors lead to persistence. Although *T. gondii* tissue cysts could be frequently found in the eye, brain, muscle, and cardiac tissue, it is unknown whether the parasite shows tropism toward a particular cell type or which cell lines are more permissive for long-term residence.[7]

5 | CONCLUSION

Toxoplasmosis is an infection that affects all warm-blooded animals. Felids as both definitive and non-definitive hosts are one of the most important animals in the transmission of toxoplasmosis. It is possible to detect the presence of *T. gondii* in the body fluids of infected animals. Both alterations of the metabolic pathways and dysregulation of amino acids and lipids might provide an insight into the detection of toxoplasmosis. Analytical techniques such as MS coupled with either LC or CE, NMR, and Raman spectroscopy have potential in the determination and detection of toxoplasmosis in a relatively short time compared to the conventional methods of analysis. However, there are a number of limitations associated with metabolic dysregulation at different stages of the infection. Therefore, further investigation of metabolites is needed.

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