Comprehensive metabolomics of the experimental opisthorchiasis

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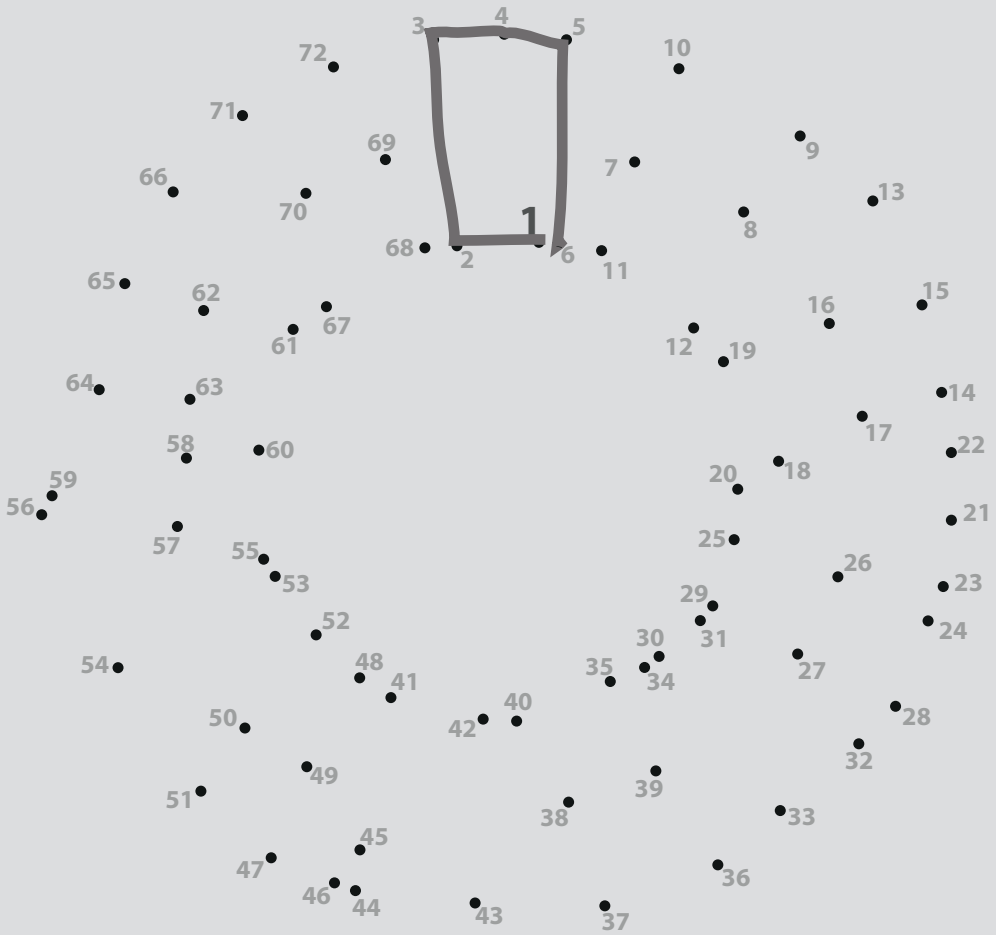
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General introduction

Opisthorchiasis: a brief overview

Food-borne trematodes are one of the big groups of human helminthiases, includes liver, lung, and intestinal flukes. They affect more than 56 million people around the world and 900 million at the risk [1, 2]. These parasites are mostly prevalent in developing countries with tropical climate: Asia, Africa, and South America [1]. The flukes of *Opisthorchiidae* family are responsible for the largest "share" of infected population with approximately 46 million people infected and 600 people – at risk. *Opisthorchis viverrini* (*O. viverrini*), and *Clonorchis sinensis* (*C. sinensis*) have tropical and subtropical endemic areas such us Thailand and China, respectively [3, 4]. However, food-borne trematode *Opisthorchis felineus* (*O. felineus*), which belongs to the same family, has another unique prevalence area around the middle and lower Ob and Irtysh rivers (Western Siberia) where average annual temperature is +1 ^{o}C. *O. felineus* was described for the first time at the end of the XIX century in the Northern Italy as a parasite of the cats. In 1891, the Russian scientist K. N. Vinogradov described this parasite in the human liver during post-mortem examination and named it the "Siberian liver fluke" [5]. In the last decade of the 20th century (1990-2000) up to 80% of population were infected with *O. felineus* in the Siberian region [6]. Today, despite availability of the affordable treatments and relatively straightforward diagnostics, the infection still impacts the Russian health care system (Figure 1). Furthermore, the cases of *O. felineus* infection are being reported in the Far East, Southeast Asia, and Eastern Europe and well as in North America, which may be attributed largely to global mobility or migration [7].

All three species of *Opisthorchiidae* have endemic areas around rivers or lakes, which is due to a complex life cycle of the flukes. There are two intermediate hosts; the first intermediate host is a susceptible snail, and the second – a fish of *Cyprinidae* family. In the first intermediate host, parasite eggs develop from miracidia to cercariae through sporocysts and rediae. Then metacercariae enter the second intermediate host – freshwater fish, where the cercaria develop to metacercaria. After eating contaminated undercooked fish, the parasite enters the final host (human or other fish-eating mammals). When the metacercaria

Figure 1. Numbers of reported new cases of O. felineus infection per 100 000 population per year in Russia in 2011-2013 (the figure was generated using, the data summarised by Fedorova O.S. et al., [6])

reach the duodenum of the final host, they ascend through the ampulla of Vater into biliary ducts (their final destination), where they become adults and start to pass eggs from 3rd to 4th week post-infection [8].

A clinical presentation of opisthorchiasis is not very specific; neither for acute, nor for chronic forms of the disease. For example, the main symptoms of the acute phase such as fever and abdominal pain, are strongly dependent on the intensity of infection and, previous exposure to the parasite, commonly, opisthorchiasis with worm burden less than 100 flukes is asymptomatic, and nonspecific mild symptoms are associated with 100 to 1000 worms [9]. Yet, with a typical, for a *Trematoda*, life span of 25-30 years, the flukes obstructing the bile ducts of the host may trigger multiple local and systemic complications due to the bile duct obstruction and usually presented in a form of fibrosis, cholangitis, obstructive jaundice, hepatomegaly, abdominal pain, and nausea [3]. Importantly, epidemiological and animal studies show that *O. viverrini* and *C. sinensis* infections can lead to cholangiocarcinoma (bile duct cancer), which has resulted in the classification of these parasites to the Group I carcinogens by the International Agency for Research on Cancer [10]. Discovery of an association between opisthorchiasis and an increased risk of cholangiocarcinoma has triggered

interest in the disease as evident from the number of publications overtime (Figure 2). Most of the publications cover the common areas of parasitology and epidemiology. Yet, in the last twenty years the pattern of opisthorchiasis research has changed; studies on diagnosis,

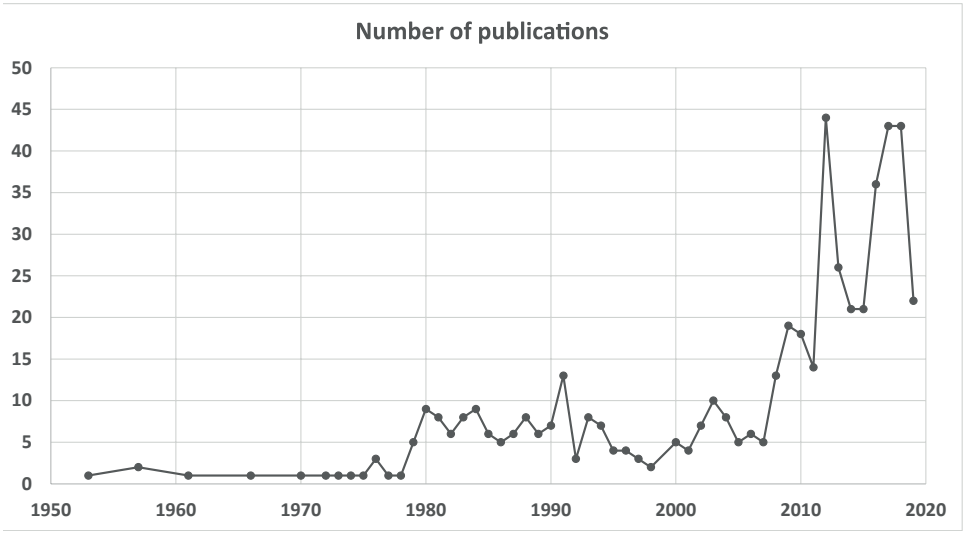

Figure 2. Dynamics of the publications on opisthorchiasis based on an online bibliographic database "Web of Science"

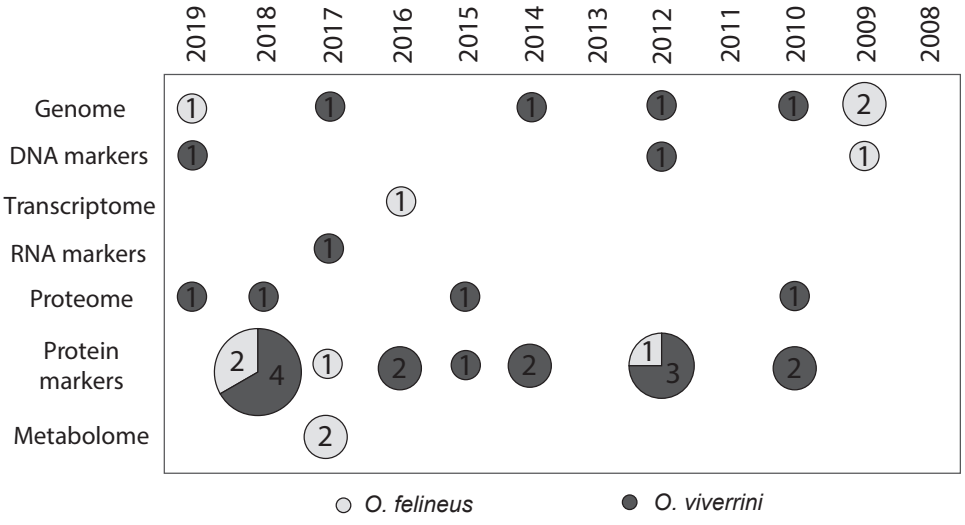

Figure 3. The frequency of the publications on opisthorchiasis related to "omics" sciences; light-grey – O. felineus, dark-grey – O. viverrini

treatment, and additionally, opisthorchis-inducted cholangiocarcinoma started to be performed taking new molecular-based approaches.

New, enabling molecular and analytical techniques are used more and more frequently to generate data, which describe the parasite and its interaction with its host at a molecular level (Figure 3). Therefore, genomes of *O. viverrini* and *O. felineus* have been well studied [11-14]; genomes of the three species (*O. viverrini*, *O. felineus*, *C. sinensis*) have been compered [14] and, the mitochondrial genome of *O. felineus* was sequenced [15]. At the same time, DNA markers of oxidative stress, such as 8-oxo-7,8-dihydro-2'-deoxyguanosine, were discovered as significant markers that predict the initiation, promotion and progression of opisthorchiasis-induced cholangiocarcinoma [16, 17]. The transcriptomic study of *O. felineus* has obtained four highly encoded proteins myoglobin, vitelline proteins, cathepsin F, and 28 kDa glutathione S-transferase which participate in adaptation of the fluke in the bile duct [18, 19]. One of important study was exploring and comparing transcriptome of *O. viverrini* and *C. sinensis* [20], which also generated data on differences between transcriptome of juvenile and adult stages of *O. viverrini* [21]. Most notable are differences among the C13-peptide and cathepsin L-like cysteine peptidases, which play key roles in tissue migration, immune evasion and feeding, and, thus, represent potential drug and/or vaccine targets [21].

Proteomics is probably the best studied area when it comes to biology of *Opisthorchiidae*. For instance, the excretory/secretory products of adult stage of *O. viverrini* were shown to include proteins that have been associated with cancers, including proteases with different characteristics, orthologues of mammalian growth factors and anti-apoptotic proteins [22]. Moreover, it has been shown in animal models that a liver antioxidant enzyme, peroxiredoxin 6, plays a key role in host response to the infection and be both a biomarker and a therapeutic target for opisthorchiasis [23]. In addition, the studies at different "omics" levels, have allowed integration of results transcriptomics and proteomics. The identification of cathepsin B-1 protease as a possible serodiagnostic antigen was guided by transcriptomic data and confirmed by excretory/secretory proteome analysis of *O. viverrini*. Also, a recombinant form of cathepsin B-1 protein was produced and tested as a serodiagnostic antigen in enzyme linked immunosorbent assays that showed a sensitivity and specificity 67% and 81%, respectively [24]. There are also a few protein markers of

cholangiocarcinoma detectable during chronic opisthorchiasis: plasma exostosin 1, liver 14-3-3-eta, cadherin-related family member 2 and lysosome associated membrane glycoprotein 1 and 2 in urine, provide the basis for development of novel diagnostic biomarkers [25-27].

However, the metabolome is not investigated at all, apart from 2 publications that are part of this thesis [28, 29]. It is important to have a full overview of an infection at multiomics level and therefore data on the metabolome of the parasite and changes in host metabolism are still needed. To address this need, the aim of our studies is to fill the existing data gap; we concentrate on the contemplation of risks associated with the *O. felineus* infection using metabolomics. The hypothesis is that the parasite remodels host metabolism, which leads to the development of the associated pathologies.

Thesis outline

Metabolomics is a post-genomic discipline that offers the researcher a combination of advanced analytical techniques capable of simultaneously detecting multiple compounds and the multivariate data modeling. In this way, the physiological status or "metabolic phenotype" of an organism can be represented as a combination of metabolite concentrations/abundances in biofluids. This thesis is focused on application of the metabolomics to study experimental opisthorchiasis.

Chapter 2 presents a critical overview of the current status, merits, and limitations of metabolomics in helminthology. The comparison of the published results was systematically assessed for the first time. Animal studies on trematode infections where NMR spectroscopy was used were included, to allow cross-comparison of the results published in different laboratories.

Chapter 3 and **Chapter 4** are focused on exploratory NMR-based metabolomics study of biofluids in O. felineus-associated opisthorchiasis. **Chapter 3** is dedicated to the metabolic changes detected in urine samples; **Chapter 4** covers the changes upon infection in blood plasma samples. Both sets of samples (urine and plasma) were collected within the same study, which was designed as a longitudinal study with two levels of infection intensity. The studies had allowed us to analyze metabolites associated with development of the infection in time, gender-specific reaction to the infection and response to the different infection burden.

Chapter 5 is focused on the development of a method for the NMR-based metabolic profiling/phenotyping of stool samples, the method is then used in **Chapter 6**.

Chapter 6 is a quantitative NMR metabolomics study of experimental opisthorchiasis which describes metabolic changes at the chronic stage of infection. The unique feature of this study is that we describe a metabolic landscape of the infection using a combined analysis of the host's body fluids and the most relevant tissue samples. We show which data blocks/samples are most affected by the infection and provide an optimal combination of the metabolites which could be considered as a signature of infection.

Finally, **Chapter 7** consists of a general discussion of the principal findings.

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