

RESEARCH ARTICLE

Serum Antibody Ig G and Ig M Titers for *Opisthorchis felineus* Correlate with Eggs in Faeces - a Comprehensive Study in Chuvash Republic, Russia

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Abstract

The Cholangiocarcinoma is a. The risk of development of cholangiocarcinoma, generally a rare type of a liver tumor, increases during infection of Opisthorchiasis. For this reason the timely detection of Opisthorchiasis is important for Cholangiocarcinoma prevention. There are many studies which concern the detection of pathogenesis of *Opisthorchis viverrini* infection but a little known about *Opisthorchis felineus*. In this study we investigate a correlation of the eggs which are found in a faeces and are comparable with a serum Ig G and Ig M antibody level that were detected with ELISA test in a large group of patients. The result is showing positive correlation between evidence of the *Opisthorchis felineus* eggs that were found in a faeces and antibody Ig G and Ig M level in a serum. Moreover the combination of two methods can improve the Opisthorchiasis diagnostic: the serum antibody and faeces investigation of eggs .

Keywords: The *Opisthorchis felineus* - eggs number - serum Ig G - Ig M - ELISA

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Introduction

It was proved that the Opisthorchiasis infection was associated with the cholangiocarcinoma (Thane et al., 2015). A helminth is one of the species of the opisthorchiasis infections that affect an hepatobiliary distal duct, a gall-bladder and a pancreatic duct. For this reason the timely detection of the Opisthorchiasis is important for preventing the cholangiocarcinoma and the prevention of spreading the infection. The disease of the Opisthorchiasis causes two main species: *Opisthorchis viverrini* with the main areas of distributions in Asia and *Opisthorchis felineus* (*O. felineus*) with the main geographical distribution in Russia and other European countries such as a Germany (Ogorodova et al., 2015). There are three main methods to diagnose this infection: a faeces investigation for eggs, an examination faeces with PCR method, and an immunological investigation of blood samples with ELISA (Rucksaken et al., 2015). PCR methods are very time consuming and expensive among all these methods. For this reason ELISA is still widely used as the best screening method.

The Chuvash republic is a region with a high density population (67.5 person per square kilometers in 2015) but with low opisthorchiasis disease rate. One of the reasons is a low occurrence of opisthorchiasis in Chuvashia as it is detected at the early stage of the disease, which

may prevent spreading of the parasite, as it was shown in Thailand (Suwannahitorn et al., 2013; Thaewongiew et al., 2014). The main method of opisthorchiasis diagnostic in Chuvash republic is the ELISA test. In a literature there are several controversial papers which concern the correlation between anti *Opisthorchis* antibody level and number of eggs in faeces, but all this studies were investigated on *Opisthorchis viverrini*, but not on *Opisthorchis felineus* (Elkins et al., 1991). For this reason, we decided to explore the *Opisthorchis felineus* incidence rate in Chuvashia with a high density in population, but a low level of opisthorchiasis and to find a correlation between the eggs quantity in a faeces with the data from the laboratory ELISA test.

Materials and Methods

The total number of investigated patients was 25738 of adults with the age range between 18-86 years old. Patients took the test for helminthes eggs in 2012-2014 in the Hospital of the Chuvash republic. The eggs were diagnosed with Kato-Miura technique which is used for detection *O. felineus* infection. (Saengsawang et al., 2013). For the serological study for detection of the specific Ig G antibodies 4603 patients were investigated with ELISA for *O. felineus* specific Ig M and Ig G antibodies (Assay kit, ZAO Vector-Best, Novosibirsk, Russia).

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Table 1. Faeces Examination for Eggs

Year	Men	Women	Men & Women
2012	14	26	40
2013	8	6	14
2014	12	8	20
Total	34	40	74

Table 2. The ELISA Test Results for Anti-Opisthorchis Antibodies

Year	Men	Women	Men & Women
2012	10	11	21
2013	16	18	34
2014	12	18	30
Total	38	47	85

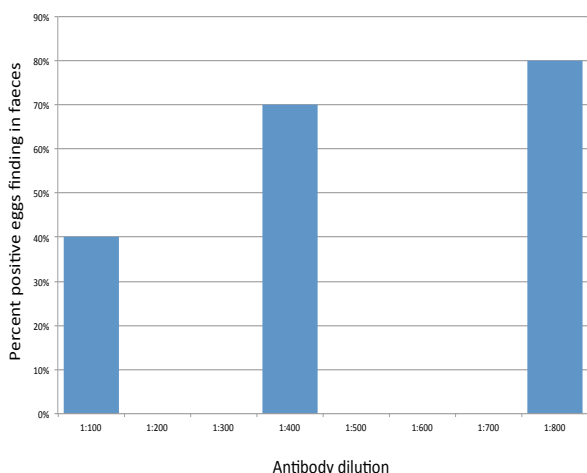


Figure 1. The Correlation between an Antibody Dilution Detected with ELISA and Intensity of the Infection. (Percent Positive Eggs finding in faeces), p- value > 0.05

Statistical analyses

All groups of patients were assessed with the different estimates with using 1-way ANOVA test (Bonferroni multiple comparison test) and at p-value equal at less or 0.05 consider as significant.

Results

The total number of patients with *O. felinus* eggs found in faeces shown in the table 1. The Spreading of infection in adult patients was low and the total amount was 74 from 25738 examined patients. However, in 10 patients with positive ELISA, the eggs were detected after one or two times re-examination. ELISA was performed on 4603 patients with 85 positive results (Table 2).

In 10 % seropositive patients Ig G the antibody level was 1:800, other seropositive patients had the antibody level from 1:100 to 1:400. 29 patients with the positive results for Ig G and Ig M (39% from all the seropositive patients) after ELISA carried out a faeces examination for eggs. During examination *O. felinus* eggs in faeces were detected only in 40 % of the cases in seropositive patients with Ig G and Ig M with 1:100 level, if patients had the quantity of 1:400-1:800 and the eggs were found in 70 % and 80 % correspondingly (Figure 1).

Discussion

In this study for the first time a positive correlation was shown between *O. felinus* antibody level and eggs in faeces in a large number of patients. Before the comparable results were shown of the *O. viverrini* infection (Elkins et al., 1991; Watwiengkam et al., 2013). Also the combination of two methods was found: the faeces study for eggs with Kato-Miura technique and ELISA for *Opisthorchis* specific antigens, it had the direct correlation relationship which can improve *O. felinus* diagnostic results (Starostina and Paniushkina, 2014). Levels of antigen in the serum significantly correlated with number of *O. felinus* egg per gram. The faeces were shown in several studies but there are small numbers of patients that lead to controversial results with positive correlation or negative results (Akai et al., 1995; Watwiengkam et al., 2013).

These results can possibly show the similarity in pathogenesis and immune response between both species *O. viverrini* and *O. felinus*. The detection of the *Opisthorchiasis* by MAb-ELISA ELISA is sensitive and specific and there is more sensitivity than microscopic faeces examination for eggs (Sirisinha et al., 1995). There are not only serums but also a salivary IgG *O. viverrini* antigens that can be used for the *opisthorchiasis* infections detections (Chaiyarit et al., 2011). The Immune response for the *O. felinus* is more associated with the somatic p105 and three egg an antigen proteins, such as p74, p70, p64 and less with excretory and secretory 20, 11, 25 antigens proteins (Chaiyarit et al., 2011). Animals were infected by the *opisthorchis* eggs production was coincided with the IgG appearance (Nockler et al., 2003). A strong immune response against the *opisthorchiasis* is associated by the increasing antigens as the parasite was maturing (Sripa and Kaewkes, 2000). An Immunological response to liver fluke antigens was also correlated with the severe liver ultrasonography findings, including the cholangiocarcinoma. (Akai et al., 1994). An increasing level of Ig G occurs when observed of hepatobiliary disease complication and when the cholangiocarcinoma is developing (Pinlaor et al., 2012).

The number of eggs in faecal consistency and the worm related with the burden in human, can be change to the during treatment, in particular the praziquantel treatment, in first few days number of eggs increased per gram feces and then it decreased to very low number in a several days . (Bychkov et al., 1990; Sithithaworn et al., 1991). In animals in the hamster eggs production of the *Opisthorchiasis* also positive correlate with the worm size (Flavell et al., 1983).

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