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MULTIPLEX PCR TEST FOR DIFFERENTIATION OF OPISTHORCHIASIS AND METORCHIASIS

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Opisthorchis felineus (O. felineus) and *Metorchis bilis (M. bilis*) are members of the *Opisthorhidae* family and pose a serious problem in veterinary and human medicine. According to various sources, approximately 600-750 million people worldwide are at risk of infection with opisthorchid worms [1]. *O. felineus* and *M. bilis* have similar morphological features at the larval stage, similar geographic distribution, and virtually the same life cycle. However, *O. felineus* and *M. bilis* infect different organs of the definitive host and cause different parasitic diseases, for example, *O. felineus* parasitizes the liver and causes opisthorchiasis, while *M. bilis* is localized in the gallbladder and its ducts and causes methorchiasis [2].

In our country, the diagnosis of "opisthorchiasis" is made regardless of which species of the *Opisthorchiidae* family infected the animal or person. In veterinary and medical practice, when diagnosing opisthorchiasis, a standard method is used – coproscopy and duodenal intubation [3]. These methods are based on microscopic examination of biological material for the presence of pathogen eggs. However, the cyclical nature of helminth egg production and the uneven

distribution of eggs throughout the contents of the large intestine lead to a false negative result [4].

Diagnosis of opisthorchiasis by clinical symptoms is difficult due to the absence of symptoms and syndromes characteristic only of this disease. For accurate diagnosis, it is necessary to develop a highly sensitive and specific PCR test [5].

Using of molecular genetic methods for identification and differentiation of *O. felineus* and *M. bilis* can solve a number of problems in the diagnosis of opisthorchiasis. PCR also allows one to identify O. *felineus* and *M. bilis* at any life stage (egg, miracidium, metacercaria, marita).

The aim of this work is to develop a multiplex PCR test that will differentiate opisthorchiasis and methorchiasis.

In this work, specific primer pairs based on the mitochondrial *COX1* gene were developed. The detection limit of the multiplex assay was estimated by serial dilutions of genomic DNA from the samples. The multiplex PCR identifies *O. felineus* and *M. bilis* in a single reaction mixture using specific fragment sizes for *O. felineus* 307 and *M. bilis* 252 bp. The lowest DNA concentration detected was 100 pg for *M. bilis* and *O. felineus.*

The main advantage of the developed PCR test is the absence of cross-reactions, the ability to detect mixed invasion in one test tube and examine a large number of samples at one time. PCR also allows detecting opisthorchid pathogens at different stages of development, including metacercariae living in carp fish.

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