ერთხელ 60 დღის განმავლობაში დოზებით 1/10, 1/100 და 1/1000 დლ$_{50}$, რაც შეადგენდა 20 მგ/კგ, 2 მგ/კგ და 0,2 მგ/კგ მასაზე. საკონტროლო ჯგუფის ვირთაგვები (n=10) იღებდნენ სასმელ წყალს. ვირთაგვები, ნატრიუმის ფთორიდის დოზის მიხედვით, დაიყო ჯგუფებად, თითოეულში – 10 ცხოველი; მაჩვენებლების შეფასება ხორციელდებოდა მე-10, მე-20, 30-ე, 50-ე და მე-60 დღეს. თავისუფალრადიკალური პროცესების ინდუცირება ნატრიუმის ფთორიდით დასტურდებოდა სისხლის შრატის ქემილუმინესცენტური რეაქციით, დიენური კონიუგატების რაოდენობა ღვიძლის ქსოვილის ჰომოგენატებში შეფასდა სპექტროფოტომეტრულად, ხოლო თიობარბიტურატმჟავას რეაქტანტებისა - მალონური დიალდეჰიდის და თიობარბიტურატმჟავას რეაქციით. დადგენილია მაჩვენებლის მომატება დოზის 1/10 და 1/100 დლ$_{50}$ შემთხვევაში ლუმინესცენციის ინტენსივობის 30-ე დღეს და მისი შემცირება მე-60 დღეს, NAD(P)-ის H-ცოტოქრომის და რედუქტაზას მომატება ღვიძლის მიკროსომულ ფრაქციაში კვლევის დასაწყისში და თანდათანობთი შემცირება 50-ე და მე-60 დღეს ორივე დოზის გამოყენების შემთხვევაში. თიობარბიტურატმჟავას რეაქტანტების დიენური კონიუგატების შემთხვევაში ექსპერიმენტის ყველა ვადაზე აღინიშნა მატების ტენდენცია. ციტოქრომ-P-450 და ციტოქრომ-b5 მომატებული იყო 30-ე დღემდე და შემდეგ თანდათანობით მცირდებოდა 60-ე დღემდე. ნატრიუმის ფთორიდის ხანგრძლივმა შეყვანამ შესაძლოა გამოიწვიოს ტოქსიკური პროდუქტების წარმოქმნა და ჰეპატოციტების მიკროსომული მემბრანის ფერმენტული აქტივობის შემცირება.

A MULTIPLEX PCR ASSAY FOR THE DIFFERENTIAL DETECTION OF *OPISTHORCHIS FELINEUS* AND *METORCHIS BILIS*

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Trematodes are parasites that have caused severe damage to human health since antiquity [1]. There are more than 91 species that infect humans and belong to 46 genera all over the world. According to their habitat in definitive hosts, they are classified as blood flukes, liver flukes, lung flukes, throat fluke, pancreatic fluke, and intestinal flukes [2]. Liver flukes belong to the family of *Opisthorchiidae* include 33 genera cause opisthorchiasisin piscivorous mammals, birds, and humans [3]. Human populations show high levels of infection with the main three liver fluke species within each of their distributional ranges [2]. Up to 680 million people worldwide are at risk of infection [4]. Recent estimates indicate that 45 million people living in Asia and Europe are infected, with approximately 35 million *C. sinensis* cases, 10 million *O. viverrini* cases, and 1.2 million cases of *O. felineus* [5-7]. The pathogen *M. bilis*, which occurs in the same territory as *O. felineus*, has attracted particular attention. It is widely registered in Russia and Kazakhstan, and there are several cases of mixed infections in humans and animals [8-13].

A variety of methods have been established for the effective diagnosis of opisthorchiasis infection, which include antigen-specific enzyme-linked immunosorbent assay (ELISA) [14-16] and various other polymerase chain reaction (PCR) technologies [17-19]. ELISA kits available on the market are not capable of the failure of *O. felineus* and *M. bilis* species detection in opisthorchiasis infection what is one of the major deficiencies. There are no commercially available molecular diagnostic kits for the simultaneous detection of mixed infections by *O. felineus* and *M. bilis*. Therefore, there is no clear understanding of the distribution of each of these species, their localization in the definitive host and approaches to treatment. The aim of this study was, therefore, to establish a multiplex PCR assay for the differential detection of *O. felineus* and *M. bilis* in clinical specimens, which will be necessary for the epidemiology, diagnoses, and control of trematodes infections.The advantage of this method of molecular diagnostics is the high specificity of the reaction, the speed of the results obtained, and the possibility of differential diagnosis of two types of pathogens.

Material and methods. *Samples collection and DNA extraction*

Samples of adult worms of *O. felineus* and *M. bilis* were collected from the artificially infected Syrian hamsters (Akmola region) and infected foxes (Karaganda region) in the territory of Kazakhstan. Genomic DNAs were extracted from adult parasites using the BioSilica DNA extraction kit (Novosibirsk, Russia), according to the manufacturer's instructions. Duodenal bile and feces samples of humans suspected of contracting infectious diseases were kindly provided by Astana Infectious Diseases Hospital, Kazakhstan, in compliance with patient confidentiality, and stored at −80°C until DNA extraction. Sample preparation was carried out according to the method of Duenngai K. et al.: a sample (feces - 500 mg, bile - 0.5 ml) is mixed with 4 ml of physiological saline and 0.4 ml of ethyl acetate, centrifuged at 4000 rpm for 10 min, followed by removal of the supernatant [20]. Genomic DNA was extracted from bile and feces samples using a method recommended by Duenngai K. et al. with some modification. The amount and purity of the extracted DNA could be determined by measuring absorption at 260 nm and 280 nm in the NanoDrop 2000 (Thermo Scientific, USA). DNA was dissolved in ddH_2O and stored at – 70°C.

Standard PCR. Fragments of co1 gene were amplified using primer pair (*OpiOpe2-co1F* 5'-TGGGGAGTTGATTTTTT-GATGTT-3' / *COI-uniRv* 5'-AGCAATAACAAATCAAGTAT-CATG-3') for both opisthorchiids in order to reveal species-specific nucleotide substitutions. The PCR product was sequenced and deposited in GenBank (MT325502 - MT325505).

Species-specific primer design. Based on the *COX1* sequences, genome DNA from *O. felineus* and *M. bilis* were designed the multiplex PCR primers by targeting conserved sequences flanking variable regions with online free available primer programs PerlPrimer v1.1.21 (http://perlprimer.sourceforge.net) and Oligo Analyzer 1.2 software (http://www.genelink.com). Details of primer pairs are presented in Table 1.

Table 1.Forward and reverse primers used in the multiplex PCR for O. felineus and M. bilis

Species	Primers	Sequences 5′-3′	Products (bp)
O. felineus	*CO1nOf-F*: *CO1nOf-R*:	5′-TTGGAATGATTAGTCATGTTTGTACG-3′ 5′-CCCCACCTATAGTAAAAAGCACTAT-3′	307
M. bilis	*COInMb-* *F*:*COInMb-R*:	5′-TGTTAATATTGCCGGGGTTTG-3′ 5′-TTTATCCCAGTAGGAACACCTATAAC-3′	252

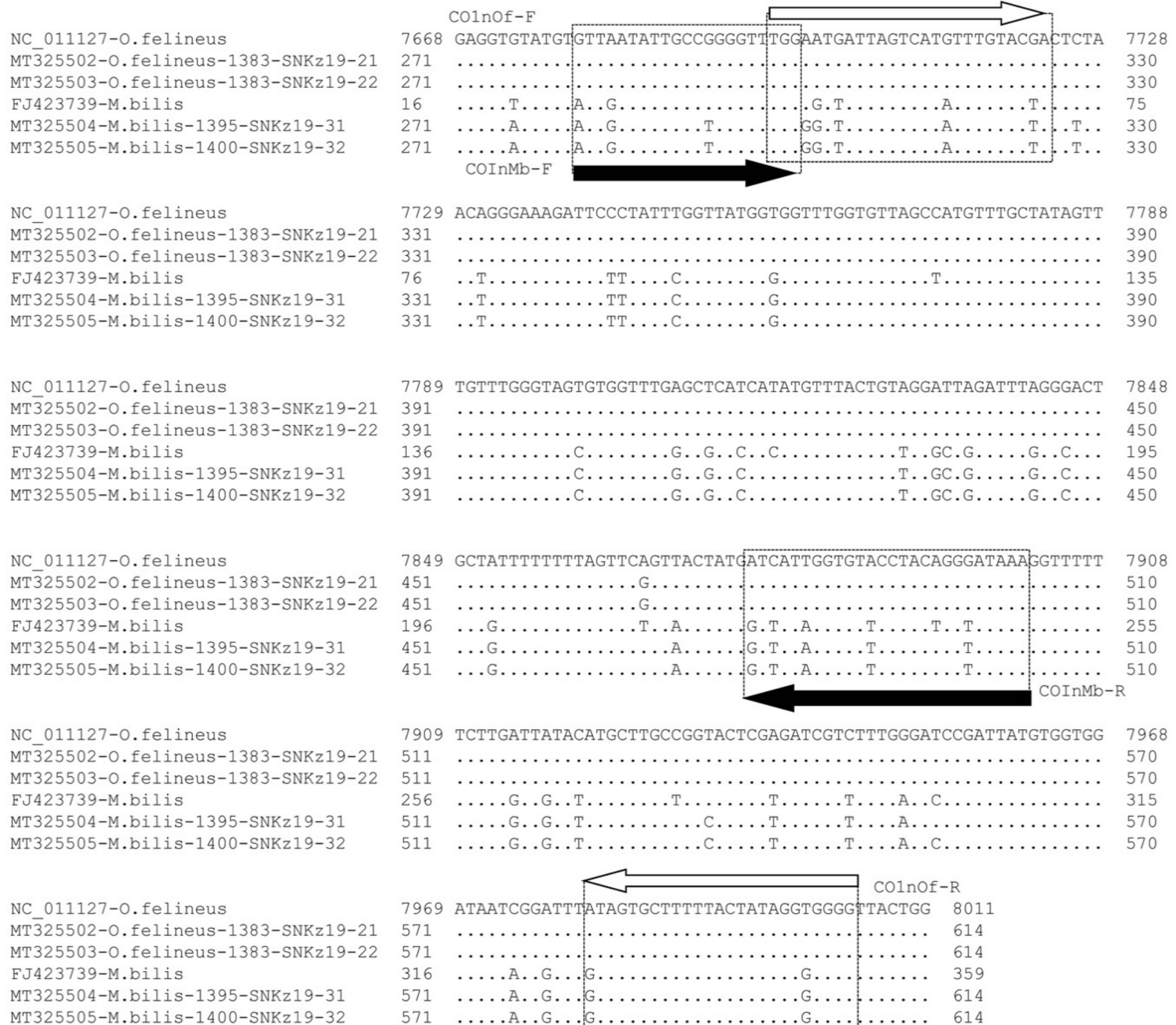

Fig. 1. COX1 fragments alignment used for primers design

Separated PCR and the standard conditions. The PCR parameters were optimized, and the reaction was carried out in a final reaction volume of 25µl containing 1$^{\times}$ Hot Start PCR Buffer (20 mM KCl, 5 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, pH 8.3), 2.5 mM $MgCl_2$ and 1 U Maxima Hot Start Taq polymerase (Thermo Scientific, USA), 2 mM of dNTP, 100 pmol of each *COX1* primer (*CO1nOf-F/R* or *COInMb-F/R*) and 30 ng extracted trematode DNA. In order to detect potential contamination, a PCR mixture with RNA/DNA-free water was regularly used as a negative control. PCR was performed as follows: denaturation at 95°C for 5 min, followed by 35 cycles with 30 s denaturation at 95°C,primer annealing at 59°C for 40 s, and extension at 72°C for 50 s with a final extension during 5 min at 72°C; and amplification products were stored at 4°C until they were visualized. PCR products (7µl) were visualized by electrophoresis in 1.5% agarose gels that were pre-stained with ethidium bromide (EB) and viewed under UV light (VilberLourmat UV-Transilluminator + with Bio-Capt software). The electrophoretic buffer solution was 1$^{\times}$TAE buffer.

Optimization of multiplex PCR primers. Optimization of the primer combinations was based on the specificity of the work of primers with DNA pathogens. The amplification of the conserved region of the partial *COX1* gene of *O. felineus* and *M. bilis* using 100 pmol of each specific primer pair (*CO1nOf-F/R* and *COInMb-F/R* primers) was carried out in a 25 µl reaction volume as described above. To examine specificity, the primers were tested together and separately with both DNA from *O. felineus* and *M. bilis*.

Optimization of multiplex PCR conditions. The multiplex PCR reaction is affected by many factors. In order to obtain the best reaction parameters, the multiplex PCR was optimized by varying single parameters while other parameters were maintained. Therefore, the parameters of the PCR assay were optimized by the varying temperature of primers annealing (56, 58, 60, 62, 64, 66°C), the concentration of dNTPs (0.05-0.5 mM) and Taq DNA polymerase

(1.0, 1.5, 2.0, 2.5 and 5.0 U) in a 25-µl reaction volume. A mixture of the genomic DNA, which contained the same amount of genomic DNA of the two types of parasites, was used as a template to amplify the corresponding target genes. The total volume of each reaction system was 25 µl, which included 1 µl of template DNA (about 15 ng of genomic DNA). PCR was performed as follows: denaturation at 95°C for 5 min, followed by 25-40 cycles with 15 s denaturation at 95°C, primer annealing at 62°C for 25 s, and extension at 72°C for 30 s with a final extension during 5 min at 72°C. After the reaction, 7 µl of the reaction solution was mixed with 7 µl of loading buffer for 1.5% agarose gel electrophoresis.

The sensitivity of the multiplex PCR assay. The sensitivity of the multiplex PCR assay was evaluated using a tenfold serial dilution method. The limit of the multiplex PCR assay detected DNA was verified using serial dilutions of mix genomic DNA of each parasite in nuclease-free water, and the final DNA concentration for each parasite in a 25µl reaction system was 10 ng/µl, 1 ng/µl, 100 pg/µl, 10 pg/µl, 1 pg/µl, respectively.

The specificity of the multiplex PCR assay. The specificity of the multiplex PCR was verified using genomic DNAs of other common worms (*Taenia spp.*, *Toxocara spp.* and *Trichinella spp.*) inhabiting the intestine of human and genomic DNAs of main bacteria (*E. coli*, *Pseudomonas spp.* and *Bacillus spp.*) found in the contents of the gallbladder.

Multiplex PCR for the detection of parasites from naturally infected samples. The Multiplex PCR was verified using genomic DNAs from human feces and bile as templates. Clinical samples were collected from the humans suspected of contracting infectious diseases that were kindly provided by Astana Infectious Diseases Hospital, Kazakhstan. The multiplex PCR assay was carried out in a final reaction mixture of 25µl, containing 2µl templates, 4µl optimal primers with 0.5µl from each forward and reverse primer (*CO1nOf-F/R* or *COInMb-F/R*), and 1 µl Maxima Hot Start Taq polymerase (Thermo Scientific, USA), followed by thermal cycling conditions and visualization process mentioned above.

The biosafety ethics for this research was approved by the Animal Ethics Committee of Veterinary Medicine Faculty of KATU (Ethical approval letter, No. 1, 09.11.2017), before commencing the project.

Results and discussion. The amplification of the genome DNA from *O. felineus* and *M. bilis* produced products of 709 bp length. The fragments ~ 345 bp were subjected to sequence analysis to design species-specific primers (Fig. 1).

Four species-specific primers produced the DNA fragments of 307 bp *(O. felineus*) or 252 bp *(M. bilis*) in PCR analysis as expected (Fig. 2).

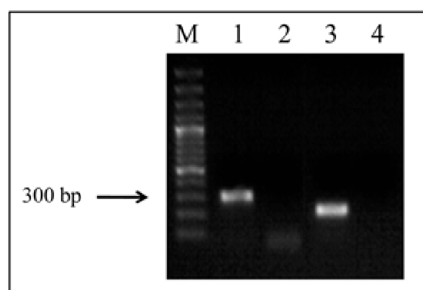

Fig. 2. Electrophoregram of a single PCR under standard conditions: Lane M, DNA ladder (bp); lane 1, O. felineus; lane 3, M. bilis; lane 2, 4, negative control

The multiplex PCR products of mixed templates of the two parasites are shown in Fig. 3. The product containing two DNA bands (307 and 252 bp) was amplified with mixed DNA templates of *O. felineus*/*M. bilis* and each specific primer pair (*CO1nOf-F/R* and *COInMb-F/R*). The results showed that the optimal annealing temperature of the multiplex PCR reaction was 60 to 62°C (Fig. 4), while the optimal dNTP and Taq Polymerase concentrations were 0.3 mM (Fig. 5) and 1.5 U (data not shown), respectively. The number of cycles largely determines the required total duration of the multiplex PCR assay. The optimal number of multiplex PCR cycles was 35, which is the standard number in this reaction (Fig. 6). In addition, the concentrations of each pair of primers were optimized, and the results showed that the optimal concentration of each pair of oligonucleotide primers was 0.1 µM (data not shown).

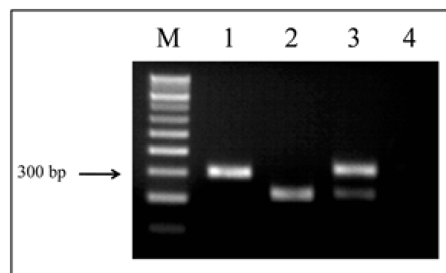

Fig. 3.Electrophoregram of multiplex PCR with both DNA from O. felineus and M. bilis: Lane M, DNA ladder (bp); lane 1, CO1nOf-F/R primers; lane 2, COInMb-F/R primers; lane 3, CO1nOf-F/R and COInMb-F/R primers; lane 4, negative control

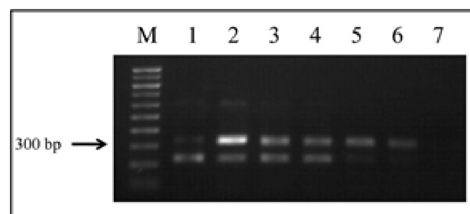

Fig. 4. Electrophoregram of multiplex PCR products in optimization of varying temperature of primers annealing conditions: Lane M, DNA ladder (bp); lane 1, 56°C; lane 2, 58°C; lane 3, 60°C; lane 4, 62°C; lane 5, 64°C; lane 6, 66°C; lane 7, negative control

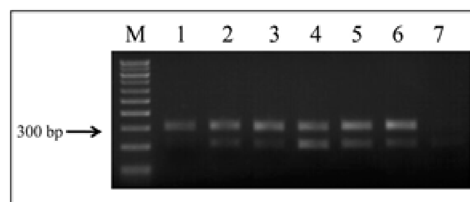

Fig. 5. Electrophoregram of multiplex PCR products in optimization concentration of dNTPs conditions: Lane M, DNA ladder (bp); lane 1, 0.05 mM; lane 2, 0.1 mM; lane 3, 0.2 mM; lane 4, 0.3 mM; lane 5, 0.4 mM; lane 6, 0.5 mM; lane 7, negative control

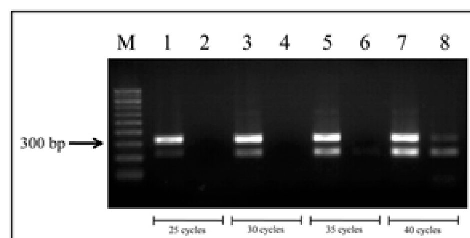

Fig. 6. Electrophoregram of multiplex PCR products in cycles optimization conditions: Lane M, DNA ladder (bp); lane 1, 3, 5, 7, positive control; lane 2, 4, 6, 8, negative control

The sensitivity of the proposed multiplex PCR assay was defined as the minimum DNA molecule concentration, which could be detected. DNA standards, which were diluted from 10 ng to 1 pg, were used for the multiplex PCR. As shown in Fig. 7, the detection limit of the multiplex PCR for *O. felineus* and *M. bilis* was 100 pg, respectively.

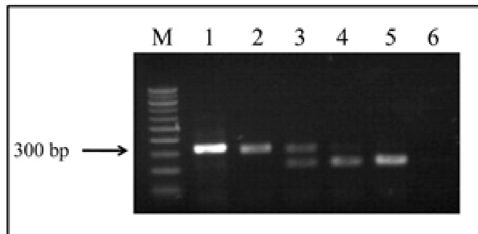

Fig. 7. Determination of the sensitivity of the multiplex PCR. Lane M, DNA ladder (bp); lanes 1-5, the concentration of each O. felineus/M. bilisDNA were 10 ng/1 pg, 1 ng/10 pg, 100 pg/100 pg, 10 pg/1 ng, 1 pg/10 ng, respectively; lane 6, negative control

In order to confirm the specificity of the multiplex PCR developed in this study, the genomes of three species of parasites (including *Taenia spp.*, *Toxocara spp.* and *Trichinella spp.*) and three bacteria (*E. coli*, *Pseudomonas spp.* and *Bacillus spp.*) were selected as the DNA template for reaction under optimized conditions (Fig. 8). The multiplex PCR test was performed with the genomes of *O. felineus* and *M. bilis* as the template DNA, which served as a positive control.

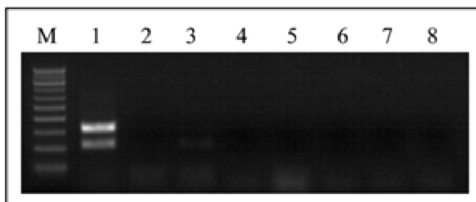

Fig. 8. Test for specificity of the multiplex PCR assay: Lane M, DNA ladder (bp); lane 1, positive control; lane 2, negative control; lane 3, Taenia spp.; lane 4, Toxocara spp.; lane 5, Trichinella spp.; lane 6, E. coli; lane 7, Pseudomonas spp.; lane 8, Bacillus spp

By developed multiplex PCR assay, a total of 9 feces and 2 human bile samples were tested. Four feces samples tested positive with a fragment size of about 252 bp identified as *M. bilis* infection (Fig. 9). One feces sample showed a double band with a fragment size of about 252 and 307 bp featuring a mix of *O. felineus* and *M. bilis* infection. Four feces samples showed a negative result. Two bile samples showed also showed a double band pointing to a mixed of *O. felineus* and *M. bilis* infection.

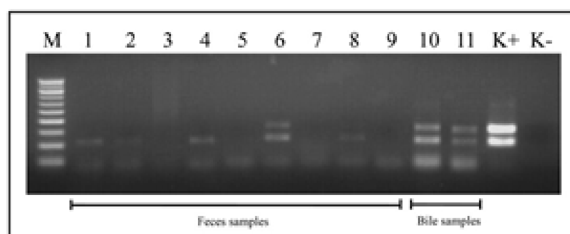

Fig. 9. The PCR results of DNA sample from human feces and bile: Lane M, DNA ladder (bp); lane 1-9, feces samples; lane 9, 10, bile samples; lane K+, positive control; lane K-, negative control

Currently, fecal examination for the detection of parasite eggs and serological studies by ELISA are the main methods for the detection of opisthorchiasis infection. However, these methods of diagnosing have some drawbacks. There are very laborious and fail to accurately distinguish the species of the pathogen [14,21-24].

Molecular methods can be an alternative to the existing methods because they have a high sensitivity and specificity, cheapness during mass screening, as well as the ability to determine the species affiliation at any life stage of the parasite. It is crucial for the sanitary-epidemiological and environmental monitoring. Primer specificity is a critical determinant of the success of a multiplex PCR assay. Pauly A. et al. reported the use of the part of the mitochondrial cytochrome c oxidase I gene for specific detection of adult specimens of the opisthorchiid liver fluke species *O. felineus* and *M. bilis* [17]. Kang S. et al. developed a fast and accurate molecular identification system for human-associated liver fluke species (*Opisthorchis viverrini*, *Opisthorchis felineus*, and *Clonorchis sinensis*) using the PCR-RFLP analysis of the *18S-ITS1-5.8S* nuclear ribosomal DNA region [19]. Brusentsov I. et al. developed multiplex polymerase chain analysis for identification of the ribosomal RNA gene cluster fragment incorporating internal transcribed spacer 2 (*ITS2*) of differentiates parasitic diseases induced by *O. felineus* and *M. bills* [18]. These are all examples of the possible use of PCR methods for the precise identification of parasites, but the methods have not been further developed, have not been commercialized, and have not yet found their practical application.

Mitochondrial genes are amongst the most popular molecular markers that have been widely used in molecular diagnoses of parasitic organisms [17,25,26]. Therefore, we explored the *COX1* genes as molecular markers to develop a multiplex PCR assay for the differential detection of *O. felineus* and *M. bilis* in clinical specimens (Fig. 1). In this study, we developed a multiplex assay that is sensitive to discriminate and diagnose two trematode parasites (*O. felineus* and *M. bilis*) simultaneously in a single reaction compare to the conventional PCR method. The specificity analysis showed that no cross-reactivity was observed between each other (Fig. 3), as well as with other worms inhabiting the small intestine [27] and main bacterial strains found in various pathologies in the bile [28] (Fig. 8).

In most cases, the sensitivity of a multiplex PCR assay will be reduced with increased numbers of target genes in the system. However, the minimum detected DNA of the proposed multiplex PCR assay was 100 pg, low enough to produce results in case of low DNA yield, which is in agreement with the results of previous studies.

For optimization of the multiplex PCR assay, varying temperature of primers annealing, the concentrations of dNTPs and Taq DNA polymerase, and, as well as the optimal number of multiplex PCR cycles, were optimized in this study. The concentrations of the primer pairs for *O. felineus* and *M. bilis* were set at 0.1 μM, and the specificity was judged as appropriate. Experimentally established that the optimal annealing temperature of the multiplex PCR reaction was 60 to 62°C, so in further experiments, we used a temperature of 62°C. dNTPs are raw materials for the synthesis of target fragments. In this study, the target fragments were all 559 bp, but two were synthesized. Therefore, under consideration of obtained results, we recommend a dNTP concentration of 0.3 mM to amplify the corresponding target fragments. Based

on experimental results and cost, the optimal concentration of Taq DNA polymerase was recommending 1.5 U per reaction. The optimal number of multiplex PCR cycles was 35, which allowed producing the required number of amplicons for good visualization of the result in the agarose gel and to avoid a false-positive result.

In addition, to determine whether the multiplex PCR assay was appropriate for the detection of pathogens in clinical samples, 9 feces and 2 human bile samples were tested. The results showed that two pathogens (*O. felineus* and *M. bilis*) were detected in the 1 stool sample and 2 bile samples with the proposed multiplex PCR assay. 4 stool samples showed the presence of the *M. bilis* pathogen. 4 stool samples showed a negative result. Accordingly, the present results indicated that the assay could be used in clinical investigation.

Conclusion. In conclusion, the multiplex PCR assay is an efficient tool for the detection, and simultaneous diagnosis of *O. felineus* and *M. bilis* trematodes from clinical specimens, the lowest limit of detectable DNA was 100 pg for two parasites. The developed method of molecular diagnostics will be used to study the spread of the O. felineus and M. bilis pathogens in humans and animals on the territory of the Republic of Kazakhstan and their role in the etiology of opisthorchiasis infection. Consequently, this essay will be potentially useful in epidemiological studies, diagnosis, and treatment of opisthorchiasis infections.

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SUMMARY

A MULTIPLEX PCR ASSAY FOR THE DIFFERENTIAL DETECTION OF *OPISTHORCHIS FELINEUS* AND *METORCHIS BILIS*

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Opisthorchis felineus and *Metorchis bilis* are two common small worms that parasitize in the gallbladder and bile ducts of the liver of humans and carnivores. These parasites have a severe impact on health and are considered pathogens of serious diseases worldwide, such as cholangiocarcinoma. However, there are still no commercially available molecular diagnostic kits capable of simultaneously detecting these parasites in humans. Therefore, the study aimed to develop a multiplex PCR analysis that will differentially determine these two opisthorchiasis infections in one reaction. Two specific primer pairs for a multiplex polymerase chain reaction (PCR) were designed based on corresponding mitochondrial genome sequences. The multiplex assay detection limit was assessed by serial dilutions of the genomic DNAs of trematode worms examined. Naturally, infected samples of human bile and feces were tested using the developed assay. A multiplex PCR assay was developed based on mitochondrial DNA that accurately and simultaneously identifies two trematode species in one reaction using specific fragment sizes of 307 and 252 bp for *O. felineus* and *M. bilis*, respectively. The optimal reaction conditions, specificity, and sensitivity of the multiplex PCR assay were investigated. The lowest DNA concentration detected was 100 pg for *M. bilis* and *O. felineus* in a 25µl reaction system. This study provides an efficient tool for the simultaneous detection of *O. felineus* and *M. bilis*. The proposed multiplex PCR assay will be potentially useful in epidemiological studies, diagnosis, and treatment of this mixed opisthorchiasis infection.

Keywords: *COX1*, *Metorchis bilis*, *Opisthorchis felineus*, multiplex PCR.

РЕЗЮМЕ

МУЛЬТИПЛЕКСНЫЙ ПЦР-АНАЛИЗ ДЛЯ ДИФФЕРЕНЦИАЛЬНОГО ОБНАРУЖЕНИЯ *OPISTHORCHIS FELINEUS* И *METORCHIS BILIS*

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Opisthorchis felineus и *Metorchis bilis* - два распространенных небольших червя, которые паразитируют в желчном пузыре и желчных протоках печени человека и плотоядных животных. Эти паразиты считаются возбудителями серьезных заболеваний, таких как холангиокарцинома. Однако по сей день не имеется коммерчески доступных наборов для молекулярной диагностики, способных одновременно обнаруживать этих паразитов у человека.

Целью исследования является разработка мультиплексного ПЦР-анализа, который позволит дифференцировать эти две описторхозные инфекции в одной реакции.

Разработаны две специфические пары праймеров для мультиплексной полимеразной цепной реакции (ПЦР) на основе соответствующих последовательностей митохондриального генома. Предел обнаружения мультиплексного анализа оценивался путем серийных разведений геномной ДНК исследуемых трематод. Инфицированные образцы желчи и кала человека протестированы с помощью разработанного метода анализа. Мультиплексный ПЦР-анализ разработан на основе митохондриальной ДНК, которая точно и одновременно идентифицирует два вида трематод в одной реакции с использованием конкретных размеров фрагментов 307 и 252 п.н. для *O. felineus* и *M. bilis*, соответственно. Исследованы оптимальные условия реакции, специфичность и чувствительность мультиплексного ПЦР-анализа. Самая низкая обнаруженная концентрация ДНК составила 100 пг для *M. bilis* и *O. felineus* в 25 мкл реакционной системы. Проведенное исследование представляет собой эффективный инструмент для одновременного обнаружения *O. felineus* и *M. bilis*. Предлагаемый анализ мультиплексной ПЦР потенциально полезен в эпидемиологических исследованиях, диагностике и лечении смешанной описторхозной инфекции.

რეზიუმე

მულტიპლექსური პჯრ-ანალიზი *OPISTHORCHIS FELINEUS*-ის და *METORCHIS BILIS*-ის დიფერენციული აღმოჩენისათვის

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Opisthorchis felineus და *Metorchis bilis* – გავრცელებული მცირე ზომის ჭიებია, რომელიც პარაზიტობს ადამიანის და ძუძუმწოვარი ცხოველების ნაღვლის ბუშტში და ღვიძლის ნაღვლის სადინარებში. ეს პარაზიტები ითვლება სერიოზული დაავადებების გამომწვევებად, კერძოდ, ქოლანგიოკარცინომის. თუმცა, დღემდე არ მოიპოვება მოლეკულური დიაგნოსტიკის კომერციულად ხელმისაწვდომი ნაკრებები, რომელიც შესაძლებელს გახდიდა ამ პარაზიტების ერთდროულად აღმოჩენას ადამიანის ორგანიზმში.

კვლევის მიზანს წარმოადგენდა მულტიპლექსური პჯრ-ანალიზის შემუშავება, რომელიც შესაძლებელს გახდის ამ ორი ოპისტორქოზული ინფექციის დიფერენცირებას ერთი რეაქციის ფარგლებში.

შესაბამისი მიტოქონდრიული გენომის თანმიმდევრობათა საფუძველზე შემუშავებულია პრაიმერების ორი სპეციფიკური წყვილი მულტიპლექსური პჯრ-სთის. მულტიპლექსური ანალიზის აღმოჩენის ზღვარი ფასდებოდა საკვლევი ტრემატოდების გენომური რნმ-ის სერიული განზავებით. ადამიანის ნაღვლის და განავალის იდენტიფიცირებული ნიმუშების ტესტირება განხორციელდა ანალიზის შემუშავებული მეთოდის გამოყენებით. მულტიპლექსური პჯრ-ანალიზი შემუშავებულია მიტოქონდრიული დნმ-ის საფუძველზე, რომელიც ზუსტად და ერთდროულად ახდენს ორივე სახის ტრემატოდის იდენტიფიცირებას ერთი რეაქციის ფარგლებში *O. feline*-ისა და *M. bili*-სთვის კონკრეტული ზომის ფრაგმენტების გამოყენებით, შესაბამისად, 307 და 252 პგ. შესწავლილია მულტიპლექსური პჯრ-ანალიზის რეაქციის ოპტიმალური პირობები, სპეციფიკურობა და მგრძნობელობა. დნმ-ის ყველაზე დაბალმა აღმოჩენილმა კონცენტრაციამ რეაქციული სისტემისათვის შეადგინა 100 პგ *M. bili*-სთვის და 25 მკლ *O. feline*-სათვის. აღწერილი კვლევა წარმოადგენს ეფექტურ ინსტრუმენტს *O. feline*-ის და *M. bili*-ის ერთდროული აღმოჩენისათვის. შემოთავაზებული მულტიპლექსური პჯრ-ანალიზი პოტენციურად სასარგებლოა შერეული ოპისტორქოზული ინფექციის ეპიდემიოლოგიური კვლევის, დიაგნოსტიკისა და მკურნალობისათვის.

BIOLOGICAL CHARACTERIZATION OF BACTERIOPHAGES AGAINST STREPTOCOCCUS AGALACTIAE

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Group B Streptococcus (GBS) or *Streptococcus agalactiae* is a Gram-positive, beta-hemolytic, catalase-negative, and facultative anaerobe coccus, which colonize the gastrointestinal and genitourinary tract [1]. GBS species are sub-classified into ten serotypes depending on the immunologic reactivity of their polysaccharide capsule [2]. *Streptococcus agalactiae* causes serious infection diseases and mostly affects immunocompromised patients with chronic diseases and newborns. Infants can be infected during birth from GBS carrier mother, either intra utero or during birth rupture of membranes, also through the inhalation or swallow of bacteria during the delivery [3]. Currently, available GBS prevention strategies which are given by CDC and its mandatory in Georgia as well will not prevent all cases of early-onset disease. *Streptococcus agalactiae* neonatal sepsis risk factors are bacterial colonization; premature birth, low weight, membrane rapture; high temperature during labor, long dry period, Urinary tract infection, etc. GBS can cause infections such as sepsis, pneumonia, and meningitis. A small number of newborns recovered from GBS infection have a long-term disability [3,4].

Antibiotics and especially Penicillin played important role in GBS prevention and treatment. But for treatment dramatically increase number of penicillin-allergic patients' antibiotic prophylaxis should be done very carefully and determine the penicillin-allergy status of all patients. Erythromycin, Vancomycin, and Clindamycin are recommended for penicillin-allergic individuals [5,6].

Uncontrolled use of antibiotics and increase number of antibiotic resistance strain renewed the interest of the modern world to the alternative antimicrobial agents as are Bacteriophages.