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ҚАЗАҚСТАНДАҒЫ «СІБІР БЕКІРЕСІ МЕН ОРЫС БЕКІРЕСІ» БУДАНДАРЫН ӨСІРУ ТӘЖІРИБЕСІ

Бұл мақалада Қазақстанның оңтүстігіндегі балық шаруашылығында «Сібір бекіресі мен орыс бекіресі» будандарының осы жылдық шабақтарын өсіру тәжірибесі жазылған. Бассейінде балық азықтылығын, осы жылдық шабақтардың орта тәулікте абсолютті және салыстырмалы өсуінің, будандардың бастапқы және соңғы массаларының көрсеткіштері келтірілген. Алынған нәтижелері Ресей ғалымдарының ұсынған нормативтік өміршеңдік көрсеткіштерімен салыстырылды.

Кілт сөздер: бекіре, бекіре буданы, сибрус, бассейінде өсіру, өміршеңділігі, балық өнімдері.

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AN EXPERIENCE OF BREEDING THE HYBRID BETWEEN SIBERIAN AND RUSSIAN STURGEON IN KAZAKHSTAN

The experience of breeding the one-years of hybrid between siberian and russian sturgeon in fish-breeding farm of south of Kazakhstan is described in an article. The parameters primary an final mass of one-years, absolute, relative and middle-day growing of one-years, the fish-productivity of reservoirs are presented. The comparison of got parameters of lively and of norm parameters which got by russian scientists, is given.

Keywords: sturgeon-breeding, hybrids of sturgeon fishes, siberian sturgeon, russian sturgeon, breeding in reservoirs, lively, fish-productivity

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POLYMERASE CHAINREACTION TO TESTSOF LEPTOSPIROSIS

Annotation. The authors performed research for optimizing conditions for polymerase chain reaction that indicates pathogenic Leptospira in isolated regions of Kazakhstan.

*Keywords:*leptospirosis, polymerasechainreaction, electrophoresis.

Introduction

Leptospirosisbelongs to zoonotic infections, which has wide spreading in the world. In the external environment leptospira parasites in natural foci on small wild mammals; in anthropurgicenvironment - on farm animals and synanthropic rodents [1,2]. According to many scientists circulation of pathogenic Leptospira in natural focus, mainly is due to feature and duration of leptospiras on mammals, in which the infection becomes a chronic process, accompanied by sustained release of Leptospirawith urine [3,4,5,6,7] . A distinctive feature of leptospirosis in modern times is lack of clinical manifestations but it is being followed by leptospiras[8].

AccordingtoWHOexperts [1967] inEuropemostly can be found such serotypesas Pomona, Tarassovi, Icterohaemorrhagiae, Canicola, Grippotyphosa, Australis, Bataviae, lessBallum, Javanica, AutumnalisandCynopteri. In Asia all serotypes can be found except Cynopteri, in Australlia - Pomona, Tarassovi, Australis, Icterohaemorrhagiae and Grippotyphosa, on American continent – Pomona, Hardio, Tarassovi, Wolffi, Canicola and Icterohaemorrhagiae.

According to local researchersleptospirosis of different animals is distributed in all regions of the Republic and etiological structure of leptospirosis is represented in most leptospirosisby pomona, icterohaemorrhagiae, tarassovi, canicola, hebdomadis, australlis, sejroe, and grippotyphosa[8,9,10,11,12].

Having learnt the fact that in many cases the disease is asymptomatic [11, 12 , 13] , today it is possible to find meat products of animals infected with leptospirosis in the markets, that is why its important to develop methods of performing express control the carcasses and internal organs of slaughtered animals. Microscopy is not sensitive enough as it allows to findleptospira when their number contains hundreds of thousands of microbial cells in cm^3. The usage of screening selective nutrient things requires a lot of time the average duration of analysis takes 10-15 days. In the condition of modern producing it it needs to be operative, that's why we are not to be in doubt about actuality of searching and being adapted on new methods of express-analyzing. It is important to note that mostly demanded thing nowadays is molecular-genetic methods which shows to study DNA and RNA cells of micro organs which exist in the animals that needs to be estimate sanitary-hygiene condition of of slaughter livestock products with high techniques.

Availabilityofbasicresearchofleptospiranucleicacidsandalso work on genetic-systematicresearchoftheseorganismsallows to do the result aboutthepossibilitiesofusingmethods of biotechnologytosolvethe problemsassociatedwithleptospirosis [14, 15 , 16].

Leptospiras are refered to microorganisms with the features of phenotypic and genotypic polymorphism [17]. There are cases when even a single nucleotide replacements in the nucleotide sequences of the target genes of the pathogen is likely to disturb the primers'annealing process, ultimately , leading to false results of PCR . Therefore there is a legitimate question: whether it is possible to use PCR test systems which were elaborated on the basis of leptospira gene strains from one region of the worldby studying leptospira infection in other regions [18].

In connection with the above the aim of this work was to use PCR analysis to indicate pathogenic leptospira in isolated regions of Kazakhstan.

Materials and methods of research

The research was performed in 2012 – 2013y.y. in antibacterial biotechnology laboratory of Kazakh National Agrarian University, in educational - research and diagnostic laboratory of Kazahstan-Japan Innovative Centre and in the laboratory of Kazakh Scientific Centre of Quarantine and Zoonotic infections named after Aykymbayev.

In this research, we used 9 museum strains which belong to 6 sero-groups of "historic" collection of antibacterial biotechnology laboratory:L.pomona, L.icterohaemorrhagiae, L.tarassovi, L.canicola, L.hebdomadis, L.australlis, L.sejroe, L.grippotyphosa. Leptospireswere cultivated in water-serummediumattemperature 28 ° C.

Isolation of nucleic acids from a tested material was carried with the help of a set of "DNA -sorb B" by using an inorganic sorbent.

By isolating DNA from leptospira with the help of a set of "DNA – sorb B" in order to optimize the allocate more concentrated DNA we used the following method of preliminary processing culture like that:2 ml of Eppendorf culture was added into the tubes, centrifugated for 5 minutes at 8000 rpm, the supernatant was drained and washed with TES lysis buffer which was addedin a volume of 1 ml into

the tube withprecipitate.The sediments were split on vortex , suspend it and again centrifuge it for 5 minutes at 8000 rpm. This process was repeated oncethenaccording to the instructions in the tube with previously processed culture we add 300 mcl lysis solution and resuspended it for 1 minute on a vortex then tubes are set into a programmed thermostat for 5 minutes at 65^0C, centrifuged at 5000 rpm for 5 seconds. Then we carefully poured upper layer transferring it to a new tube and added 25 mcl of universal resuspendedsorbent, mixed on vortex and placed into a rack for 2 minutes at 37^0C. The sorbent was precipitated by centrifugation at 5000 rpm for 30 seconds. Then the supernatant was removed by using a vacuum sucker.

We added 300 mcl of solution into tubes for 1st wash, and shifted it on vortex then let the sorbent sediment and removed liquid that was above sedimental layer. Then we added 500 mcl of solution for 2nd wash, mixed on vortex, centrifuged and removed supernatant. The procedure of washing with a solution for 2nd wash was repeated. Then the tubes were placed into a thermostat at temperature 65^0C for 5-10 minutes for drying the sorbent. In test tubes we added by 50 mcl of TE buffer for DNA exclusion andresuspendedit on vortex, and placed into thermostat at 65^0C for 5 minutespermanentlyshaking it on vortex. Then centrifuged it at 12,000 rpm for 1 minute. Then the supernatant contains the purified DNA which is transferred to separately labeled test tubes , and samples are readyforanalyzing.

In order to preparelysisbufferTES (100ml) we add 1.2 g. of Tris to 80 ml of distilled water,we align pH till 8.0 by adding approximately 400 mcl HCL. Then we add 4 ml of 0.5 M EDTA, 0.0585 g. NaCLand get volume to 100 ml of distilled water.

Work has been doneon the selection ofappropriateprimer pairsfor the experiment.Selection of oligo-nucleotide sequence primer pairs LEP21/LEP22 was performed on the basis of published results of analysis of gene's the nucleotide sequence, encoding the outer membrane lipoprotein synthesis of leptospiras LipL32 RM52 krischneri (HaakeD.A. Etal. 2000 , GenBank-AY461915). Selection of primers was made by computer program OLIGO 4.0. Synthesis of primers was performed by fosfoamitid method on automatic DNA synthesizer / RNA " H6 » (K &ALaborgeraete, Germany) in the laboratory of Kazakh Scientific Center of Quarantine and Zoonotic infections named after Aikymbayev. Concentration of primers have been selected experimentally (10 to 30 pmol) .

Primer annealing temperature range was determined by the given program OLIGO 4.0. ± 3 - 5^0C .

For polymerase chain reaction we used amplifier Eppendorf (Germany) of «Mastercyclernexus» series.

Results of research

The main criterionin the methods of extracting DNA is high degree of purifying nucleic acid from cellular DNA and proteins. Isolated genomic DNA must be unfragmented as it serves like a matrix for synthezing a specific product. The quality and quantity ofisolated DNA in this manner was detected on 0.8% agarose gel (Figure 1).

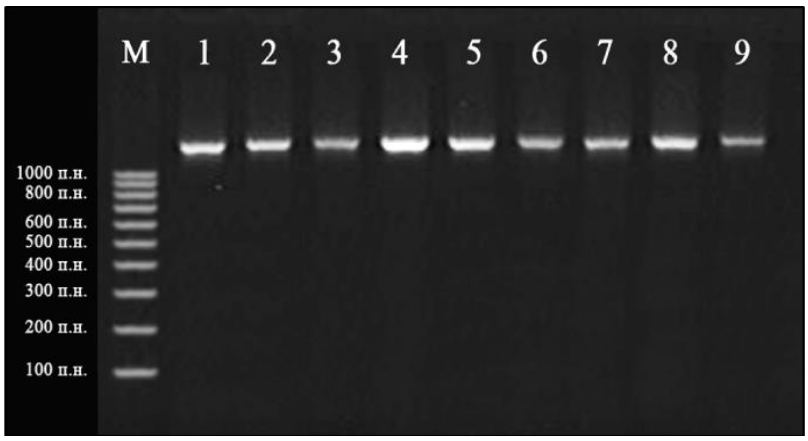

Figure 1. Electrophoregram ofDNAleptospira. Lane M is a DNAmarker which isreestered with a restriction enzyme into fragments with the known molecular weight. The lanes perform the samples of DNA which are marked from different strains of leptospiras:
1- L.pomona, 2- L.icterohaemorrhagiae, 3-L.tarassovi, 4-L.canicola, 5-6-L.hebdomadis, 7-L.australlis, 8-L.sejroe, 9-L.grippotyphosa.

The obtained results indicate the given samples of DNA molecules.

Polymerase chain reaction was performed on a thermocyclerEppendorf (Germany) series «Mastercycler nexus». Of the various options PCR conditions , the strongest signal obtained by followingManufacture provided : the first step - denaturation at 94^0c - 3 min , the second step - denaturation at 94^0 C - 30 s, annealing of primers - 60^0S - 30 seconds and extension at a temperature of 72^0C - 30 sec. - A total of 35 cycles. The final synthesis at 72^0C with a duration of 5 minutes.

The reaction mixture had the following composition: 2.5 ul of PCR buffer 50 mM KCL, 0,75 l MgCL2 (1.5mM) 0.5 l Taq platinum polymerase (5 U / ul), 0.5 ul 0.2 mMdNTP mixture concentration 17 5 l of deionized water, 2 microliters of DNA.
By studyingLeptospiraceae group members with primers LEP21/LEP22, the gene fragment coding the synthesis of lipoprotein LipL32 was found in pathogenic representatives of L.pomona, L.icterohaemorrhagiae, L.tarassovi, L.canicola, L.hebdomadis, L.australlis, L. sejroe, L.grippotyphosa (Figure 2).

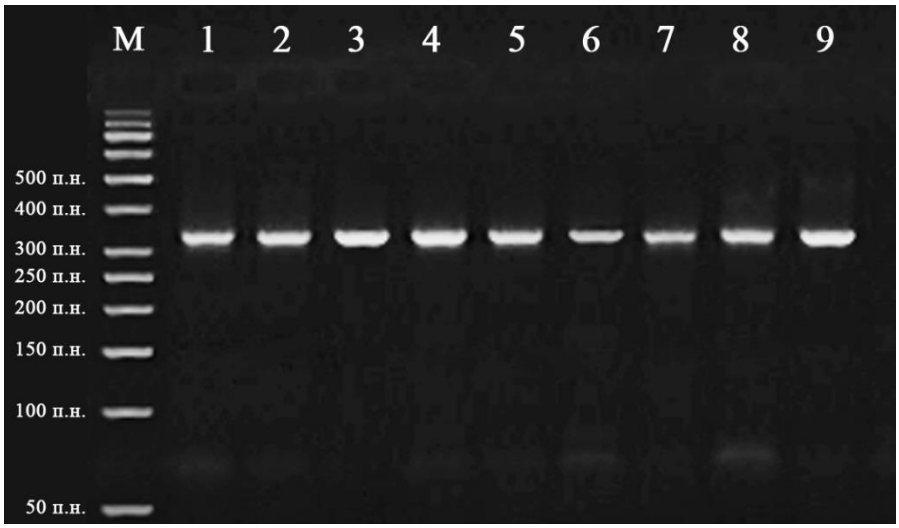

Figure 2. Electrophoregramproducts of PCR by using primers LEP21/LEP22 of DNA parasitic leptospiras. M-Marker of molecular weight with fragments of DNA known by these sizes: 50, 100, 150, etc. nucleotide pairs (n.p.).: 1- L.pomona, 2- L.icterohaemorrhagiae, 3-L.tarassovi, 4-L.canicola, 5-6-L.hebdomadis, 7-L.australlis, 8-L.sejroe, 9-L.grippotyphosa.

In figure 2 we can see that on the lanes 1-9 the stripes appearwith the size of 331 n.p. . The given fact allows us to conclude that in all tested samples by the method of PCR with subsequent detection of electrophoresis the following DNA was exposed - L.pomona, L.icterohaemorrhagiae, L.tarassovi, L.canicola, L.hebdomadis, L.australlis, L.sejroe, L.grippotyphosa.

Thus, the test results show that the given conditions of testing the polymerase chain reaction can detect pathogenic leptospira in some areas of Kazakhstan.

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ОПТИМИЗАЦИЯ МЕТОДА ВЫДЕЛЕНИЯ ДНК ЛЕПТОСПИР ИЗ СУТОЧНЫХ КУЛЬТУР

Авторами статьи проведена работа по оптимизации условий проведения полимеразной цепной реакции для индикации патогенных лептоспир выделенные в регионах Казахстана. В работе использовали 9 музейных штаммов, относящихся к 6 серогруппам из «исторической» коллекции лаборатории противобактериозной биотехнологии: L.pomona, L.icterohaemorrhagiae, L.tarassovi, L.canicola, L.hebdomadis, L.australlis, L.sejroe, L.grippotyphosa.

Ключевые слова: лептоспироз, полимеразная цепная реакция, электрофорез.

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ТӘУЛІКТІК ӨСІНДІДЕН ЛЕПТОСПИРА ДНҚ-ын БӨЛІП АЛУ ӘДІСІН ЖЕТІЛДІРУ

Мақала авторлары Қазақстан аймағындағы патогенді лептоспираларды анықтау үшін полимеразды тізбекті реакция қоюды жетілдіруге байланысты жұмыстар жүргізді. Зерттеуге бактериозға қарсы биотехнология зертханасы қорынан 9 мұражайлық штамм пайдаланылды: L.pomona, L.icterohaemorrhagiae, L.tarassovi, L.canicola, L.hebdomadis, L.australlis, L.sejroe, L.grippotyphosa.

Кілт сөздер: лептоспироз, полимеразды тізбекті реакция, электрофорез

УДК 619:616.33/. 34-008.711.2

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СРАВНИТЕЛЬНЫЕ РЕЗУЛЬТАТЫ ИММУНОГИСТОХИМИЧЕСКИХ ИССЛЕДОВАНИЙ КЛЕТОЧНОГО СОСТАВА ТИМУСА КРУПНОГО РОГАТОГО СКОТА

Аннотация. С помощью гистологических и иммуногистохимических методов с использованием Polyclonal Rabbit Anti-Human T cell, CD 3, Monoclonal Mouse Anti-Human Myeloid/Histocyte Antigen, Clone MAC 387 и Monoclonal Mouse Anti-Proliferating Cell Nuclear Antigen, Clone PC10 антисывороток, были выявлены и демонстрированы места локализации Т- лимфоцитов, макрофагов и митоза лимфоидных клеток в парафиновых срезах тимуса крупного рогатого скота, фиксированное в формалине.

Ключевые слова: крупный рогатый скот, тимус, иммуногистохимия, поли- и моноклональные антисыворотки, Т-лимфоциты, макрофаги, митоз лимфоидных клеток.

Введение

По исследованию вопросов морфологии тимуса крупного рогатого скота в странах СНГ имеется ряд научных работ [1-4].

Гатин И.М. (1990) исследовал морфогенез и клеточный состав тимуса у бычков разного возраста при откорме в индивидуальном секторе и в промышленных условиях. Состав и соотношение клеточных элементов тимуса бычков при откорме в индивидуальном секторе отражают антигеннезависимые изменения. Морфология тимуса 16-месячных бычков при откорме в промышленных условиях характеризуется большим разнообразием клеток, наличием значительного числа гранулоцитов, тканевых базофилов с выраженной деградацией, резким огрубением ретикулярных волокон стромы, мукоидным набуханием стенок сосудов микроциркуляторного русла. Выявленные изменения являются морфологическим эквивалентом возникновения аллергических реакций гиперчувствительности замедленного типа **[1].**

Применение кормовой добавки препарата "Привес-40" нормализовало морфологию тимуса, т.е. соотношение лимфоидных клеток коркового и мозгового вещества в сторону увеличения их количества в коре долек тимуса. При этом произошло снижение содержания эозинофильных гранулоцитов и тканевых базофилов.