антиденелерді турацты ендіретін ірі цара лейкоз вирусы жэне эфирдшп 2 х 107 M^{-1}-ден 4 х 108 M^{-1} дейін бірігуін байцадыц.

РЕЗЮМЕ

Представленный ретроспективный анализ данных ветеринарных лабораторий республики по эпизоотической ситуации по лейкозу крупного рогатого скота за 2002-2015 годы показал, что инфицированность животных вируса лейкоза крупного рогатого скота в среднем по стране составляет 3,0%, с колебаниями по областям от 0,12% до 6,0%, при исследовании скота к общей численности поголовья в среднем 16,12%. Основной причиной этого является неполный охват животных к серологическим исследованиям и отсутствие отечественной тестсистемы для иммуноферментного анализа. В современных условиях основу лабораторной ранней прижизненной диагностики лейкоза крупного рогатого скота, на которой опирается система профилактических мероприятий, составляет серологический метод исследования - реакция иммунодиффузии в геле агара и метод иммуноферментного анализа, с помощью которых определяют инфицированность стад вирусом лейкоза. Иммуноферментный анализ, основан на иммунологической реакции воздействия антиген-антитело и применении в качестве индикатора этой реакции маркированных ферментами моноклональных антител или антигенов. Для чего нами иммунизацией мышей концентрированным антигеном вируса лейкоза крупного рогатого скота получены лимфоциты, продуцирующие специфические антитела и слиянием их с миеломными линиями клеток Х63-Ag8.6.5.3, клоны гибридных клеток, из них выявлено один штамм, стабильно продуцирующие моноклональные антитела с молекулярной массой 51 и 24 кДа к вирусу лейкоза крупного рогатого скота и эфирностью от 2х 107М-1 до 4х 108М-1.

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EXTRACTION OF PASTEURELLA MULTOCIDA gDNA FROM ORGANS AND TISSUES OF ANIMALS USING VARIOUS METHODS

Abstract

To date, researchers have a fairly large set of methods for extracting and purifying DNA, and these methods continue to be improved and modified with reference to new research objects. In connection with the variety of living objects, there are no universal methods of DNA isolation. The use of this or that method of DNA isolation is dictated, firstly, by the specificity of the material being studied, and secondly, by what purpose is pursued: the production of total, nuclear, chloroplast DNA or other of its preparations. The DNA isolation method should be relatively simple, well reproducible and allow for the rapid production of sufficient quantities of satisfactorily purified DNA preparations. The yield of DNA depends on the nature of the starting material and is due to the content of DNA in this tissue, as well as the presence and nature of impurities that interfere with DNA purification.

In the article, based on literature sources and own research, the results of studies on the selection of the optimal method for isolation of *Pasteurella multocida* DNA from biological material are presented.

As a result of the selection of the optimal methods for isolation of *Pasteurella multocida* DNA from biological material, it was determined that all methods of DNA extraction used in the work are quite acceptable for the extraction of *Pasteurella multocida* genomic DNA.

Keywords: DNA, sorbent, Pasteurella multocida, polymerase chain reaction.

The appearance of the polymerase chain reaction (PCR) method was due to certain achievements of molecular genetics, primarily the decoding of the nucleotide sequence of the genomes of a number of microorganisms. Simplicity of performance and unsurpassed indicators of sensitivity and specificity brought unprecedented popularity to the new method. With the speed of lightning, the method spread throughout the world. PCR is used for scientific and practical research. But first of all the method has found wide application in the field of microbiological diagnostics. Currently, the PCR method is automated, fairly simple in execution and available to any molecular biology laboratory [1].

When diagnosing pasteurellosis of animals using PCR, the main working material is Pasteurella multocida [2].

Deoxyribonucleic acids (DNA) are a universal source of information on all genetic traits of any kind.

In the modern world, it seems possible to extract DNA from absolutely any available biological material, consisting of cells that have, among other things, a decorated core in their structure. These are the so-called eukaryotic cells. These include cells of animals, plants, viruses or microorganisms [3].

The main criterion in the methods of DNA isolation is a high degree of purification of the nucleic acid from the impurities of cellular DNA and proteins. The isolated genomic DNA must be unfragmented, since it serves as a template for the synthesis of a specific product [4].

Treatment of clinical material and isolation of nucleic acids is the first and most important stage of molecular biological research. The main task of this stage is to obtain a purified DNA preparation for the subsequent amplification reaction.

Materials and methods. The research was carried out on the basis of the biotechnology laboratory of the Research Institute of Biotechnology and Nature Management of the NPJSC «Zhangir Khan West Kazakhstan agrarian-technical university». Several methods of DNA isolation were used to select the optimal variant.

DNA extraction was carried out using commercial DNA sorb complexes B, and for this purpose an automatic Thermo Scientific King Fisher and a hot lysis method were used.

Isolation of DNA with the help of sorbents. A 1,5 cm^3 daily culture was added to the tubes and centrifuged 8,000 rpm for 5 minutes. The supernatant was drained and washed with a buffer solution. A TES buffer solution was added to the tube with a precipitate of 1 cm^3 - the precipitate was vortexed. Again precipitated by centrifugation 8,000 rpm - 5 minutes. Then, the supernatant was discarded and 1cm^3 of 2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid (TES) buffer added again, vortexed and 300 pl of lysing solution (iazamidine-i-thiotinad) was added and vortexed and allowed to stand for 5 minutes in a thermostat at 65 ^{0}C. Turbidity indicates a lysis. Then the sorbent - 25 pl was added, pre-shaken, after addition also mixed on a vortex and left at 37 ^{0}C for 2 minutes. Then they were shaken for a few seconds on the vortex and again put for 5 minutes at room temperature. Centrifuged 5 thousand/rpm, 30 seconds. Then, the supernatant was removed, leaving the pellets on the bottom. Then, wash solution No.1 was added with 300 pl (heated to 60-65 ^{0}C). Then centrifuged for 30 seconds at 5 thousand / revolution per minute.

The supernatant was removed and 500 pl of buffer № 2 was added. Vortexed. Then centrifuged for 30 seconds, the supernatant was removed. Then it was dried in a thermostat with an open lid for 5 minutes at 65^{0}C. Then add 50 pl TE for elution and shake vortex and leave in the thermostat for 5 minutes at 65^{0}C. It was then centrifuged at 12,000 rpm for 1 minute. Then they transferred the DNA into a test tube.

Isolation of DNA from the daily pasteurella culture using an automatic nucleic acid extraction station - Thermo Scientific King Fisher. The «Thermo Scientific King Fisher Cell and Tissue DNA Kit» was prepared according to the dialing protocol. The basis of the set is paramagnetic quartz particles, which bind the nucleic acid in the presence of chaotropic salts. After the binding step, the DNA / magnetic particle complex was thoroughly washed in washing buffers to remove all remaining contaminants and then the purified DNA was immersed in an eluent buffer.

The culture cell suspension in the Phosphate Buffered Saline (PBS) buffer was washed, then treated with a lysis buffer (containing 10 pl of P-IU per 1000 pl of buffer), so that the final concentration was 1x10^6 cells / 100 pl. Then went to the protocol set.

The isolation of DNA from the bacterial culture Pasteurella multocida was carried out by hot lysis method. Two ml of 18 h of the pasterelle broth culture was precipitated by centrifugation at 10,000 rpm for 10 minutes. The precipitate was washed with a sterile PBS buffer solution (10mMTris Hcl,

10mMKcl, 10mM Mgcl2.2mM Ethylene Diamine Tetraacetic Acid (EDTA)) twice and resuspended with 30 pL deionized water and boiled for 10 minutes. After boiling, the samples were placed in a layer of ice for 30 minutes. The samples were then centrifuged at 3000 rpm for 10 minutes to precipitate. The supernatant was stored at -20 ^{0}C until use.

Phage DNA, treated with Hind III (DNA Molecular Weight marker II (0,12-23,1 kbp)) was used as a control. Documented the results through a transglyuiluminator, using the computer program «LabWorks 4.0», determined the molecular weights of the restriction, the sum of all the restriction fragments.

The development of specific DNA sections was carried out in a thermocycler with a gradient of temperatures Mastercycler gradient, Eppendorf.

Electrophoresis of DNA amplification products of Pasteurella multocida was carried out in a horizontal electrophoresis apparatus «G-100», firm «Pharmacia», at a voltage of 8 V/cm. Electrophoresis in 2,0% agarose gel was used to record the results.

Preparation of working electrophoresis buffer. 25 cm^3 of concentrated Tris-borate buffer (TBE) were poured into a graduated cylinder, brought to 500 cm^3 with distilled water, the cylinder was closed with parafilm and mixed.

Agarose from one bottle was poured into a glass flask made of heat-resistant glass for 250 cm^3. 100 cm^3 of the working buffer was poured, stirred by rotating the flask and melted in a microwave oven until the agarose was completely dissolved, (1,5 minutes at 800 W), agarose was brought to a boil for another 1,5 minutes. The flask was removed from the microwave oven and agarose cooled, rotating the flask, to 65-70 ^{0}C.

The molten gel was poured into the mold of the chamber. Set the combs at a distance of at least 3 cm from each other. The thickness of the gel is about 6 cm.

After the gel completely solidified, the combs were gently removed. Place the substrate with the finished gel in the chamber. The chamber was filled with a ready buffer so that it covered the gel by 5 mm.

Documentation of the obtained results was carried out through a transgelilluminator. Subsequent analysis of the results was carried out using the Digi-Doc-It program.

The «DNA Wide Range marker» and «DNA Ladder 1kb» from «Sigma» were used as a marker of molecular weights.

Results of the study and discussion. The first method is based on the dissolution of DNA in the presence of a sorbent. As a result of treatment with a lysing solution, the destruction of cell membranes and the release of DNA occur. Dissolved DNA binds to the sorbent particles, and other components of the lysed clinical material remain in solution and are removed by sorbent precipitation by centrifugation followed by washing.

A second method of extracting DNA from bacteria involves the use of paramagnetic quartz particles that bind the nucleic acid in the presence of chaotropic salts. After the binding step, the DNA/magnetic particle complex is thoroughly washed in the wash buffer to remove all remaining contaminants and then the purified DNA is immersed in the eluent buffer.

We tested a method for the isolation of DNA by the hot lysis method of a pasteurella agar culture on a water bath, described by W.L. Araujo, D.A. de Angellis, J.L. Azevedo «Direct RAPD Evaluation of Bacteria without Conventional DNA Extraction» [5]. This method is very simple in execution and can be used for routine diagnostic work. The yield of genomic DNA in this method was 60-80%.

The main criteria for the development of optimal methods were the concentration and purity of the drug.

After isolation of Pasteurella multocida DNA by the above methods, a qualitative and quantitative analysis of the sample was carried out. Electrophoresis was performed in a 0,8% agarose gel in TAE buffer. The ratio between the optical densities at 260 and 280 nm was spectrophotometrically measured. The maximum absorption for nucleic acids is recorded at a wavelength of 260 nm. The preparation of DNA is considered free of impurities at a ratio of $E_{260}/_{280}$ equal to 1,8 and higher. The preparation of DNA is considered free from impurities at a ratio of $E_{260/280}$ equal to 1,8 and higher. If this is below this, the sample is contaminated with proteins or phenol.

Conclusion. Samples of Pasteurella multocida DNA, using sorbents, proved to be of low quality. The relationship between the optical density at wavelengths of 260 and 280 nm averaged 1,65 - 1,7,

which indicated contamination of DNA with protein and other impurities. Thus, the obtained results showed ineffectiveness of these methods.

Good results were obtained by fixing DNA on a magnetic particle and a silica base in a centrifuge tube.

The ratio of the optical density (E260/E280) of the obtained Pasteurella multocida DNA preparations had an average value of 1,81±0,04 (n=4).

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ТҮНІН

Кайрп тацда зерттеушшердщ жетерлштей ецбектервде ДНЕ-ын тазалау және экстракциялау эдютершщ Үлкен жиынтыгы бар, оган цоса бул эдютер зерттеулердщ жаца нысандарына сэйкес езгерюке енш дамуды жалгастыруда. ТҮрлі пршшк иелерше байланысты нацты эмбебап ДНЕ-ын белш алу эдістері жоц. ДНЕ белудщ нацты бір немесе басца эдютерш цолдану, ец алдымен, зерттелетш материалдыц ерекшелтне байланысты және екшшщен, цандай мацсатца багытталган: жалпы, ядролыц, хлоропласт ДНЕ немесе оныц басца да препараттарын ецщру. ДНЕ белу эдю салыстырмалы тҮРде царапайым болуы керек, жацсы ендірілетін және жеткшкп тҮрде тазартылган препараттарды тез алуга мҮмкіндік беруі керек. ДНЕ-ныц таза белшш шыгуы бастапцы материалдыц сипатына және зерттеліп отырган улпаныц цурылымына, сондай-ац ДНЕ-ныц тазартылуына кедергі кел^ретш цоспалардыц бар болуына байланысты.

Мацалада езіндік зерттеулер және эдебиет кездері негізінде биологиялыц материалдардан Pasteurella multocida ДНК^-ын белудіц тиімді эдістерін тацдау бойынша зерттеу нэтижелері келтірілді.

Биологиялыщ материалдардан Pasteurella multocida ДНК^-ын белудщ тиімді эдіст'ерін тандау нэтижесі бойынша жумыс барысында Pasteurella multocida геномды ДНК-ын экстракциялау Үшін ^олданылган барлыщ эдістер ^олайлы екендт анышталды.

РЕЗЮМЕ

К настоящему времени в арсенале исследователей имеется довольно большой набор методов экстракции и очистки ДНК, причем эти методы продолжают совершенствоваться и модифицироваться применительно к новым объектам исследования. В связи с разнообразием живых объектов универсальных методов выделения ДНК не существует. Использование того или иного метода выделения ДНК диктуется, во-первых, спецификой изучаемого материала, а во-вторых, тем, какая преследуется цель: получение суммарной, ядерной, хлоропластной ДНК или других ее препаратов. Метод выделения ДНК должен быть относительно простым, хорошо воспроизводимым и давать возможность быстрого получения достаточных количеств удовлетворительно очищенных препаратов ДНК. Выход ДНК зависит от природы исходного материала и обусловлен содержанием ДНК в данной ткани, а также наличием и характером примесей, препятствующих очистке ДНК.

В статье на основании литературных источников и собственных исследований приведены результаты исследований по выбору оптимального метода выделения ДНК Pasteurella multocida

из биологического материала.

В результате выбора оптимальных методов выделения ДНК Pasteurella multocida из биологического материала, определили, что все использованные в работе методы выделения ДНК вполне приемлемы для экстракции геномной ДНК Pasteurella multocida.

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THE CONTENT OF VITAMINS AND MINERALS IN MEAT OF HEALTHY AND SICK PIGS WITH CIRCOVIRUS INFECTION

Abstract

This article compares the contents of vitamins and minerals in meat of healthy and sick pigs with circovirus infection. The nutritional value of meat depends on the quantitative ratio of water, protein, fat, the content of essential amino acids, polyunsaturated fatty acids, vitamins, micro- and macroelements, and organoleptic indices of meat. Vitamin B_1 in the body is very necessary for proper metabolism. Vitamin B_1 in the meat of a sick animal was 0,68 mg, and in the control group - 0,81 mg.Vitamin E improves the function of muscles and gonads. It is present in the composition of vegetable oil, in seeds of walnut, legumes and corn, and vegetables. Vitamin B_2, like other vitamins is necessary for normal growth, it is involved in biological processes. Vitamin B_2 is 0,23 mg in the control group, 0,14 mg in the experimental group.Vitamin E in the meat of a healthy animal was 0,52 mg, and in the meat of a sick animal, 0,39 mg. Vitamin PP is necessary for carrying out the processes of biological oxidation in the body. In sufficient quantities in the liver and kidneys, yeast, meat and milk, as well as in peas, large beans, in the composition of grain flour. Vitamin PP was 3,86 mg in the control group, 3,11 mg in the experimental group.

Keywords: pig, vitamins, minerals, microelements, nutritional value, circovirus infection.

In recent years, in connection with the active development of pig production, diseases that had not previously been registered in Kazakhstan are of great importance. A particularly significant problem in industrial pig farms was circovirus infection of pigs [1].

With circovirus infections, known clinical symptoms, such as:

- depletion (multisystem exhaustion syndrome after weaning piglets from the sow);
- dysfunction of the kidneys and skin (dermatitis and nephropathy piglets syndrome (SDPP));
- a violation of the function of the respiratory tract (depending on the meaning) (proliferative and necrotic pneumonia (PNP) [2].

The nutritional value of meat depends on the quantitative ratio of water, protein, fat, the content of essential amino acids, polyunsaturated fatty acids, vitamins, micro- and macroelements, and organoleptic indices of meat.

Microelements constitute a very small part of the rations of animals. However, they play an extremely important role in the metabolism of animals, while having a significant impact on their health and productivity. Being associated with enzymes, hormones and vitamins, they affect the basic vital processes of the body, and also support the permeability of cell membranes, tissue respiration and intracellular metabolism. Therefore, the development of a rational system for feeding mineral substances and vitamins to animals is possible only taking into account the latest achievements in the field of the theory of mineral and vitamin metabolism [3].

The aim of our scientific work is with equal indices the content of vitamins and minerals in the meat of healthy and sick pigs with circovirus infection.

The research was carried out in the laboratory of the RSE «Scientific Research Institute for Biological Safety». From both groups (experimental, control group of 10 heads) from 100 g of pork samples were taken using the generally accepted methods of veterinary and sanitary examination, the composition of pork vitamins determined the device and m and HPLC and a spectrometer.

Micro-macro elements were determined with the help of the device «Atomic Adsorption