Батыс Қазақстан облысы, Зеленов ауданының шаруашылықтарында варроатоз ауруы кең тараған. Бал ара варроатозының орташа инвазиялық экстенсивтілігі 10,08 % - ды құрайды. Бал ара варроатозына кезіндегі жоғары тиімділікті апидез көрсетті. Оның экстенсивтілігі 100%. Препаратты қолданудан кейін барлық ара ұяларындағы тәжірибелік топтарда клиникалық жағдайда ауытқулар болмады.

РЕЗЮМЕ

Варроатоз – наиболее распространенное заболевание, вызываемое клещом *Varroa destructor* и протекающее круглогодично. Клещ наносит большой экономический ущерб, который складывается из гибели расплода, взрослых рабочих пчел и потери продукции. Существует большое количество методов борьбы с данным заболеванием. Наиболее радикальной мерой борьбы против варроатоза пчел является применение химиотерапевтических препаратов. На сегодняшний день при борьбе с варроатозом пчел применяют химические препараты, имеющие в своем составе следующие действующие вещества: флувалинат, флуметрин, акринатрин, формамины, кумафос, органические кислоты, эфирные масла и лекарственные растения. Так же он отмечает, что, наиболее безопасными в отношении жизнедеятельности пчел являются препараты растительного происхождения. Цель наших исследований - изучение динамики зараженности пчел варроатозом в летний период и изучение эффективности акарицидных препаратов. Варроатоз на пасеках Зеленовского района, Западно-Казахстанской области наблюдается повсеместно. Средняя экстенсивность инвазии составила 10,08 %. Наибольшую эффективность при варроатозе пчел показал препарат Апидез. Его экстенсэффективность составила 100%. Все пчелосемьи подопытной группы, где был применен данный препарат, полностью освобождены от клеща варроа. После применения препаратов на пчелосемьи у пчел подопытных групп никаких отклонений в их клиническом состоянии не выявлено.

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ANALYSIS OF RESULTS OF DNA ISOLATION FROM BIOLOGICAL SAMPLES

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, but the greatest amount of DNA is extracted using commercial PureLink Genomic DNA kits

Keywords: *DNA (deoxyribonucleic acid), electrophoresis, commercial reagent kit.*

In the diagnostic laboratory that processes daily large volumes of material, automating the DNA extraction process eliminates the influence of the human factor and conducts PCR diagnostics in a timely and efficient manner [1, 2].

The main criterion in DNA extraction methods is a high degree of purification of nucleic acid from 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 [3-5].

Materials and methods. Pasteurella multocida distributes DNA from suspected bacterial cells and pasteurellosis from suspected animals and concentrates the DNA concentration by using special device Qubit 2.1, DNA extracted from biological specimens by various commercial reagents (in agarose gel electrophoresis) quality assessment of Kostanay State University named after A.Baitursynov, Department of molecular-genetic research of the Scientific Innovation Center. In the «DNA extraction area», the following protocols were performed:

I. DNA distribution diagram using DNA-extran-1 (Synthol) kit:

1. Get the sample from blood samples:

1.1 The 1.5 ml or 2 mL required test tubes were labeled according to the samples to be tested, and 300 μL whole blood was poured into all tissues.

2. Lysis of red blood cells

2.1 Put 900 μl of lysing solution No. 1 into each tube and incubated at room temperature for 10 minutes.

2.2 The mixture was centrifuged for 2 minutes to 13000 rpm.

2.3 Removal of 20 μl of the supernatant is removed from the leucocyte concentration by leaving the surface fluid. Rotated in vortex.

3. Lysis of cell nucleus

3.1 The 300 μl of leaf solution was added into the vortex by adding lecithin solution.

3.2 The mixture was incubated for cell lysis at room temperature.

4. Deposition of DNA (sediment)

4.1 Add 100 μl of precipitation solution to a leaser. Probirka's contents are spun around the vortex.

4.2 The mixture was centrifuged for 2 minutes at 13,000 rpm.

4.3 Contains a supernatant containing DNA in 1.5 ml of pure test tubes.

4.4 The mixture is centrifuged for 2 minutes to 13000 rpm. Superheated supernatant.

5. DNA defrosting and washing

5.1 400 μl was mixed several times with the addition of a wash solution and transplanted tubes.

5.2 centrifuged for 2 minutes at 13000 rpm. Superspersed superalloy.

5.3 Opening the mouthpiece, detecting it in the air or at 370 ^{0}C.

5.4 Add 100 μl of eluent solvent № 6 to the precipitate. The mixture was stirred and heated at 650C for 5 minutes until dissolved in DNA.

5.5 Saves the obtained DNA solution -20^{0}C. It can be stored for a shorter period of + 40 ^{0}C.

II. Diagram of distribution of DNA from Pasteurella multocida bacterial cultures using the Compa-GS reagent kit:

1. For the treatment of several test tubes, 150 * (N + 1) mL laundry solution and 20 * (N + 1) μl pre-treated sorbent were dispensed into the tube.

2. Add 170 μl of the ready-made mixture to each sample and turn the tube into the vortex 3-5 sec.

3. We put on a thermostat for 20 minutes at 500 ^{0}C.

4. One minute is centrifuged to 13000 rpm.

5. The surface sludge was removed.

6. Add 200 µl of detergent № 1 to the tincture and rotate in the vortex 3-5 s.
7. centrifuged for 1 minute to 13000 rpm.
8. Absorbed liquid was removed.
9. Add 200 µl of detergent № 2 to the tincture and spin 3-5 h in the vortex.
10. centrifuged for 1 minute 13000 rpm.
11. Absorbed liquid was removed.
12. Add 200 µl of detergent № 3 to the tincture and rotate in the vortex 3-5 s.
13. One minute is centrifuged to 13000 rpm.
14. Absorbed liquid was removed.
15. At 5 ^{0}C for 5 minutes we opened the lid and put it on the thermostat.
16. Add 100 µl of eluent solution to the precipitate and spin 5-10 h in the vortex.
17. We put 5 500 ^{0}C thermostat for 5 minutes.
18. One minute is centrifuged to 13000 rpm. For a long time to maintain the sample, we replaced the supernatant liquid into new test tubes.

III. Scheme of DNA separation from blood samples by PureLink Genomic DNA kits:
1. We pour 200 µl of blood into 1.5 ml of Eppandorf tube.
2. Add 20 µl of Proteinase.
3. Add 20 µl of RNAse, rotate in a vortex, and incubate at room temperature for 2 minutes.
4. Add 200 µL lime buffer to each tube, rotate in the vortex, put the thermostat at 550C for 10 minutes.
5. Then 200 µL 96-100% ethanol was added to the vortex.
6. The resulting lemon (~ 640 µl) was poured into the test tube (with a PureLink kit).
7. The seeds were centrifuged at room temperature for 1,000 rpm 10000 rpm.
8. Remove the bottom of the filled test tube and replace the top part with a new test tube in the PureLink kit.
9. 500 µl of the buffer № 1 was centrifuged for 1 minute at room temperature at 10000 rpm; and we've moved to the new test tube in PureLink.
10. Add 500 µl of buffer buffer № 2 and transfer the tube centrifugate at room temperature at a solid rate of 3 minutes and then remove the bottom part to the test tube in PureLink.
11. The upper part was replaced by 1.5 ml Eppandorf tube.
12. Add 100 µl of eluent buffer, incubated at room temperature for 1 minute, centrifugate at high speed for 1 minute, and remove the top of the test tube. 1,5 ml of Eppandorf tissue will have purified DNA.

Results of the study and discussion. In our study, we compared different commercial DNA isolation kits with Pasteurella multocida. Three samples were taken in the study.

In this experiment, DNA was isolated from 3 blood samples and bacterial cultures. By the methods used, we measured the DNA concentration in Qubit 2.1.

Table 1 - The results of DNA isolation from various commercial kits

№	Name of the Reagent Kit	DNA Cleanliness	DNA concentration, ng / µl			Average DNA Concentration, ng / µl
			Sample 1	Sample 2	Sample 3	
1	*DNA-Extran-1 (Sintol)*	1,5	1,4	1,4	1,6	1,46
2	*Reagent of Proba-GC*	1,65	1,2	2,2	1,3	1,56
3	*PureLink Genomic DNA kits*	1,8	3,6	9,3	8,1	7

After DNA isolation of Pasteurella multocida by the above methods, a qualitative and quantitative analysis of the sample was performed. Electrophoresis was performed in a 0.8% agarose gel in TAE buffer. The greatest amount of DNA is secreted using PureLink Genomic DNA kits. The

ratio of optical density (A260/A280) of the obtained DNA preparations of Pasteurella multocida was an average of 7.

The result of a qualitative analysis of the obtained DNA preparation is presented on an electrophoregram (Figure 1).

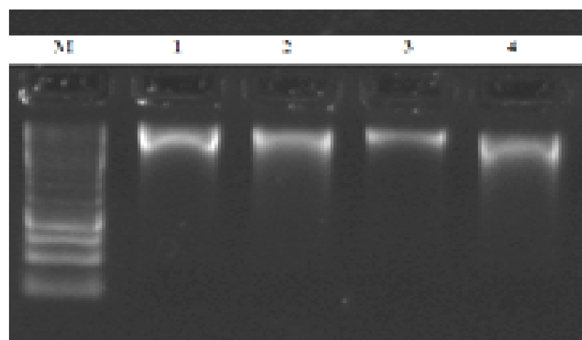

Figure 1 - DNA electrophorramhme, separated by PureLink Genomic DNA kits reagent kit: M marker, 1,2,3 - biological specimen samples, 4 - control specimens

Conclusion. We have seen that there are some of the advantages and disadvantages of the three commercials used in the research. They:

Priorities of DNA-Extran-1 (Syntol) Commercial Kit:

- Reduce the DNA fragmentation by removing the DNA from cells completely;
- Distributed DNA has high purity (A260/A280 = 1,8-1,9) and is a ready-to-use product for PCR, restriction, hybridization, and other studies.
- The kit contains no harmful ingredients, such as phenol and chloroform.

The advantages of the Commercial Kit of the Proba-GE Reagents:

- DNA has a high degree of purity;
- maximally neutralizes inhibitors, with a high content of impurities recommended for use in materials.

Disadvantages: Due to repeated washing results, DNA may be lost due to irreversible sorption in the media.

Because the PureLink Genomic DNA kits commercial kit is a distant foreign product, its contents are kept confidential.

As a result of choosing the most effective methods for DNA distribution from biological materials, it was found that all the methods used for the extraction of DNA were acceptable.

Nevertheless, the PureLink Genomic DNA kits collection had a high priority over the three commercials used during the study.

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ТҮЙІН

Қазіргі таңда зерттеушілердің жетерліктей еңбектерінде ДНҚ-ын тазалау және экстракциялау әдістерінің үлкен жиынтығы бар, оған қоса бұл әдістер зерттеулердің жаңа нысандарына сәйкес өзгеріске еніп дамуды жалғастыруда. Түрлі тіршілік иелеріне байланысты нақты әмбебап ДНҚ-ын бөліп алу әдістері жоқ. ДНҚ бөлудің нақты бір немесе басқа әдістерін қолдану, ең алдымен, зерттелетін материалдың ерекшелігіне байланысты және екіншіден, қандай мақсатқа бағытталған: жалпы, ядролық, хлоропласт ДНҚ немесе оның басқа да препараттарын өндіру. ДНҚ бөлу әдісі салыстырмалы түрде қарапайым болуы керек, жақсы өндірілетін және жеткілікті түрде тазартылған препараттарды тез алуға мүмкіндік беруі керек. ДНҚ-ның таза бөлініп шығуы бастапқы материалдың сипатына және зерттеліп отырған ұлпаның құрылымына, сондай-ақ ДНҚ-ның тазартылуына кедергі келтіретін қоспалардың бар болуына байланысты.

Мақалада өзіндік зерттеулер және әдебиет көздері негізінде биологиялық материалдардан Pasteurella multocida ДНҚ-ын бөлудің тиімді әдістерін таңдау бойынша зерттеу нәтижелері келтірілді.

Биологиялық материалдардан Pasteurella multocida ДНҚ-ын бөлудің тиімді әдістерін таңдау нәтижесі бойынша жұмыс барысында Pasteurella multocida геномды ДНҚ-ын экстракциялау үшін қолданылған барлық әдістер қолайлы екендігі анықталды. Дегенмен де, зерттеу барысында қолданылған үш коммерциялық жинақтардың ішінде PureLink Genomic DNA kits жинағы жоғары басымдыққа ие болды.

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, но наибольшее количество ДНК выделено с помощью коммерческим наборам PureLink Genomic DNA kits.