Improving the typical methods of Brucella

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Abstract. In this article the comparison of different methods for typing Brucella spp. A technique for differentsatsii B.abortus and B.melitensis based by PCR. Defined a method to more quickly typed bacteria of the genus Brucella. Polymerase chain reaction (PCR) in a short time PCR-analysis has spread around the world, quickly leaving the laboratories of scientific institutions in the sphere of practical clinical use. Diagnosis of infectious diseases, including those caused by agents that are difficult to cultivate, genotyping microorganisms, evaluation of their virulence, the definition of stability microorganism to antibiotics, gene diagnostics, prenatal diagnosis, biological control of blood – and more other the successfully PCR is used. The main stage of recovery from infectious animal diseases, including brucellosis, is timely and rapid diagnosis, which occupy an important place laboratory tests for the detection of pathogens in pathological material the main goal of the research was to determine the methods for rapid detection of Brucella spp. To solve the problem of goal were: pick primers specific to strictly Brucella spp., and optimize PCR conditions for a specific region of the genome Brucella spp. The objects of the study As objects of study were blood from three cattle and three small ruminants - sheep (n = 6), react positively to brucellosis on the results of serological tests RBP and AR. Work was carried out on the basis of the Research Institute (Laboratory of Biotechnology engineering profile) WKATU named Zhangir Khan. Polymerase chain reaction was carried out on the device company iQ5 BioRad. DNA extraction was performed by cetyltrimethylammonium bromide (CTAB). The quality of DNA extraction were determined by the method of agarose gel electrophoresis.
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Introduction

Traditional differentiation methods of various Brucella species are very laborious and time-consuming, and are not always reliable. For example, certain strains of different species of Brucella can be absolutely identical biochemical properties. Need to search for significant differences. Responsible for such differences carries the DNA of microorganisms directly. In the world of microbiology in the last twenty years have seen a revolution. This happened largely due to the rapid development and implementation in practice molecular biology techniques [1,2,3].

Research is needed due to the pressing problems of modern veterinary medicine, when it becomes apparent that brucellosis being anthropozoonosis represents a danger to livestock and humans. For this reason, research on methods to differentiate between the different Brucella species based on PCR method should be an integral part of a new, practice-oriented diagnosis of brucellosis. If we take into account the fact that the vast majority of cases of brucellosis is not subject to species differences, it becomes obvious that the only way an adequate analysis are methods of modern molecular biology, the use of which are addressed in this study [4,5].

Table 1. Types of Brucella

Types	Disease
Brucella melitensis (biovars 1-3)	Brucellosis goats, sheep, human
Brucella abortus (biovars 1-6,9)	Brucellosis of cattle, human
Brucella suis (biovars 1-5)	Porcine brucellosis, human
Brucella canis	Brucellosis dogs
Brucella ovis	Brucellosis sheep (epididymitis in rams)
Brucella neotomae	Brucellosis rats, guinea pigs, mice

In modern veterinary science and practice of detection and differentiation of pathogens brucellosis conducted using serological tests, consuming and takes a lot of time studying the culture-biochemical traits on nutrient media, where there is a need for differentiation of carbon dioxide in the formation of hydrogen sulfide, etc. To date, the known the following types of Brucella: Brucella melitensis (Maltese), Brucella abortus bovis (bovine), Brucella abortus suis (pork) and Brucella canis (dog), etc (table 1).

In recent years , molecular biology has been increasingly used PCR , DNA amplification by polymerase chain reaction (PCR) , he became one of the main methods in experimental studies to help you solve a variety of tasks in research. SA Bulat implemented genetic typing of Yersinia pseudotuberculosis strains by PCR using oligonucleotide primers 5-22 nucleotides in length , which were synthesized based on the nucleotide sequences of the hypervariable regions of the DNA of M13 phage . PCR was originally developed for the amplification and analysis of specific genetic loci, and based on the prior knowledge of the nucleotide sequences of these loci in the modified embodiment, using universal primers was suitable for a detailed analysis of the genomes of all organisms. New methods - is a necessary prerequisite for the development of any branch of science. They allow to obtain previously unavailable information , which in turn leads to a deeper understanding of the essence of the observed phenomena and stimulate further research, generating new discoveries.

PCR (polymerase chain reaction) is one of the widely used techniques of molecular biology, which is used to a significant increase in the concentration of certain nucleic acid fragments in the sample. The method is a certain copy of a DNA region with the use of various enzymes in vitro. PCR consists of three consecutive steps (denaturation, amplification and elongation).

The principle of the polymerase chain reaction (PCR, Polymerase chain reaction, (PCR) was developed by Cary Myullisom (firm "Cetus", USA) in 1983, opening the PCR has become one of the most significant developments in the field of molecular biology over the past 20 years. For Development PCR C. Myullisom in 1993 was awarded the Nobel Prize in Chemistry. Appearance PCR was due to certain advances in molecular genetics, especially decoding the genomes of a number of nucleotide sequences of microorganisms. can not say that PCR was made possible by the discovery of a unique enzyme Taq–DNA–polymerase contained the bacteria that live in geysers. Feature of this polymerase is its exceptional heat resistance (can withstand heat up to boiling temperature without loss of activity), and high operating temperatures (optimum operation - 72 °C).

Elegance, simplicity, performance, unsurpassed sensitivity and specificity of the new method yielded enormous popularity. In a short time PCR-analysis has spread around the world, quickly leaving the laboratories of scientific institutions in the sphere of practical clinical use. Diagnosis of infectious diseases, including those caused by agents that are difficult to cultivate, genotyping microorganisms, evaluation of their virulence, the definition of stability of microorganism to antibiotics, gene diagnostics and genetic fingerprinting, prenatal diagnosis, biological control of blood - this is not a complete list of areas where he successfully PCR is used.

Identification of an organism in the study usually occurs on resistance genes by the promoter as well as the genes. Distinguished: conserved genes, such sequences that vary slowly in continuing evolution. Conserved genes encode the genetic apparatus of the cell and analyzing conserved genes in organisms, we can identify the body classes. With the help of gene research can determine the average conservatism body to the family. Allocate the same genes low conservatism and highly variable genes, on them the body can be determined to species.

The aim of our research was to improve the method of differentiation of various Brucella species by the method of molecular biology PCR.

Materials and methods

Work was carried out on the basis of the Research Institute (Laboratory of Biotechnology engineering profile) WKATU name after Zhangir Khan. Polymerase chain reaction was carried out on the device company iQ5 BioRad.

As objects of study were bled from three cattle and three small ruminants - sheep (n = 6), react positively to brucellosis on the results of serological tests RBP and AR.

To differentiate types of Brucella B. abortus and B. melitensis used bacteriostatic method - differentiation in resistance to aniline dyes. For the differentiation of these cells were used, consisting of meat water, NaCl, peptone agar and dyes used as basic fuchsin and thionin 1:50 000 1:25 000.

To carry out molecular biological studies of biological samples was used typical experimental setup. Scheme of a typical experiment involves the separation of total DNA from the sample, PCR amplification of specific regions of the genome.

In the process of isolating DNA from biological samples using standard laboratory equipment for work on molecular microbiology -

automatic dispensers, centrifuges, electrophoresis equipment, visualization and documentation of gels, refrigerators, freezers, vortices, etc.

DNA extraction was performed by CTAB. The quality of DNA extraction was determined using the method of electrophoresis in agarose gel. Whole blood was taken from the animals in the test tubes with 3% EDTA based 10:1. DNA was isolated from blood using the extraction buffer consisting of 2 g of CTAB (detergent destroys cell membranes , forms complexes with proteins and polysaccharides acid), 28 ml of 5M NaCl, 4 ml 0.5M EDTA (pH 8.0), distilled water, adjusted to 100 ml. To 100 µl of blood was added 300 µl extracting buffer and incubated at 60 º C for 1 hour, the tube contents are periodically stirring. After incubation, an equal volume of chloroform, and left for 1 hour at room temperature. Tube contents were stirred continuously. Then centrifuged 5 min at 5000 rpm. The upper phase was transferred to a clean tube and add 2 /3 volume of isopropyl alcohol were mixed . The tubes with the contents was kept at room temperature for 2 hours to precipitate the DNA. Then centrifuged for 10 minutes at 12 000 rpm. The supernatant was decanted and the precipitate was washed with 70 % - ethanol. Centrifuged under the same conditions. Then, the liquid was poured over the sediment, the sediment was dried and dissolved in water -free DNA and RNA.

The main essence of the methods is that we must first remove the cell membranes, denature proteins associated with DNA, remove impurities and precipitation directly allocate already purified DNA.

Quality was determined using DNA isolated by agarose gel electrophoresis. Agarose gel electrophoresis is used in molecular biology for separation of molecules (proteins and nucleic acids, and fragments thereof) by an electric field according to their masses and spatial structure [6].

Software was used amplify DNA collection microorganisms of the genus Brucella, consisting of 42 cycles comprising 1 step "denaturation" at 95 ° C for 3 minutes, stage 2 "joke" at 63 ° C during the first minutes and 3 phase "elongation" or "fusion" at 72 ° C during the first minute. Reaction mixture consisted of the following components: the primers for PCR buffer reaction, MgCl 1.5 M, dNTP mix дизоксинуклеотидтрифосфатов and Taq-polymerase.For differentsatsii different Brucella species we have chosen the following primers: BAF and BAR strictly specific for B. abortus, BMF and BMR specific to B. melitensis [7,8,9,10].

Results of research

During the research was revealed that the blood be taken from the cow species bacterium Brucella abortus, and sheep's blood type bacterium Brucella melitensis (Table 2).

The studies determined that on nutrient media supplemented with growth characteristic fuchsine only B. abortus and B. melitensis significant characteristic growth on nutrient media with the addition of magenta and not significant on media supplemented with lauth's violet, and seeding with the blood of cattle on growth media with lauth's violet not allowed. Reading of the results was carried out after 6 days.

Table 2. Results of bacteriological differentiation Brucella

Dye	Crops from animals react positively to brucellosis in the formulation of AR and RBP	
	Cattle blood	Sheep blood
Fuchsine 1:50 000	+++	++
Lauth's violet 1:25 000	0	+

As a result of PCR using primers previously selected was established that the blood be derived from cows bacterium species B. abortus, a sheep blood bacterium B. melitensis (Figure 1).

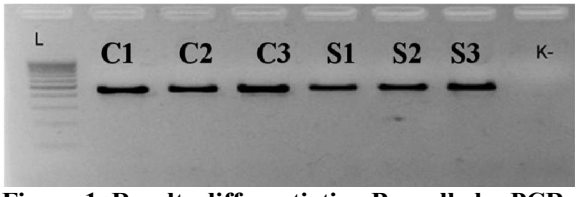

Figure 1. Results differentiation Brucella by PCR

Methodology differentiation of bacteria by PCR demonstrated its efficiency and accuracy compared to the more laborious method of cultivation of bacteria and biochemical typing. PCR can be used successfully veterinary specialists for diagnosis and differentiation of various types of Brucella.

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References

1. Acinas, S.G., R. Sarma-Rupavtarm and V. Klepac-Ceraj, 2005. PCR-induced sequence artifacts and bias: insights from comparison of two 16S rRNA clone libraries constructed from the same sample. Appl. Environ. Microbiol., 71(12): 8966 – 8969.
2. Mullis, K.B., 1987. Process for amplifying nucleic acid sequences. U.S. Patent, 4: 202..
3. Kushaliev, K.Zh., M.G. Kakishev, and B. Radojicic, 2013. Comparison of immunological changes in the body of guinea pigs after the application of two types of vaccine against brucellosis. Brucellosis in Yugoslav Europe, Veterinary Specialist Institute "Nis" Scientific-expert symposium, pp: 120-125.
4. Baily, G. G., J. B. Krahn, B. S. Drasar, and N. G. Stoker. 1992. Detection of Brucella melitensis and Brucella abortus by DNA amplification. J. Trop. Med. Hygiene 95:271–275.
5. Soltis, D. E., P. S. Soltis, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanis, V. Savolainen, W. H. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, L. M. Prince, W. J. Kress, K. C. Nixon and J. S. Farris. 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. Botanical Journal of the Linnean Society 133: 381-461.
6. Fountain, M., S.J. Weiss, A.G. Fountain, A. Shen and R.P. Lenk. 1985. Treatment of Brucella canis and Brucella abortus in vitro and in vivo by stable plurilamellar vesicle-encapsulated aminoglycosides. J Infect Dis 152(3):529-535.
7. Laemmli, U. K. 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage. Nature 227 (4): 680 — 685.
8. Morgan, W.J. and M.J. Corbel. 1976, Recommendations for the description of species and biotypes of the genus Brucella. Dev Biol Stand 31:27-37.
9. Kakishev, M.G., Kushaliev and B. Radojicic. 2013. Comparative diagnosis of brucellosis animals by PCR and ELISA. Modern integration priorities of science: from research to innovation. WKATU name after Zhangir Khan, pp: 269-272.
10. Kakishev, M.G., K.Zh. Kushaliev and B. Radojicic. 2013. Use of polimerase chain reaction (PCR) for Brucella spp. indentification and migration in the organism of guniea pig. European International Journal of Science and Technology 6 (2): 137-142.

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