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INDICATION OF BRUCELLES BY POLYMERASE-CHAIN REACTION (PCR) IN COMPARISON WITH THE COMMON METHODS OF LABORATORY DIAGNOSIS OF BRUCELLOSIS IN TURKESTAN REGION

Abstract. *The article provides an analysis of the PCR method in comparison with other methods of laboratory diagnosis of brucellosis of animals. The advantages of PCR analysis are described. The high efficiency of the polymerase chain reaction in the supervision of brucellosis infection has been shown. Among the wide variety of hybridization methods of DNA analysis, the PCR method is most widely used in clinical laboratory diagnostics. Polymerase chain reaction is a simple laboratory method of copying a DNA fragment using readily available reagents. As the number of copies increases exponentially, more than 100 billion copies can be obtained in just a few hours. copies. Currently, this method is widely used both for scientific research and for diagnostics in practical healthcare, the State Sanitary and Epidemiological Surveillance Service, epizootic monitoring, veterinary medicine (genotyping, diagnosis of infectious diseases).The method has become so popular that today it is difficult to imagine work in the field of molecular biology without its use.Recently, various modifications of PCR have been proposed, the possibility of creating a test system for detecting microorganisms, detecting point mutations has been shown,*

dozens of different applications of the method have been described, including in the diagnosis of infectious animal diseases.

***Keywords**:brucellosis, sample, PCR, DNA, brucella, indication, bacteriological method, test system, diagnostics, derivative.*

INTRODUCTION

Brucellosis is a zoonotic, mainly chronic disease of animals and humans, one of the most acute problems for modern healthcare and veterinary medicine. Being a widespread infection, brucellosis causes great economic damage to animal husbandry.

The success of the fight against brucellosis depends on the effectiveness of diagnostic studies. The possibility of making a final diagnosis for brucellosis is provided by the results of bacteriological and serological studies. Serological reactions are the basis of laboratory diagnostics. Of the serological methods currently used, the agglutination reaction (RA), the complement binding reaction (RSC), the rosebengal test (RBP).

In veterinary medicine, these reactions are widely used, since they are simple in technique, more accessible for conducting mass lifetime studies and are characterized by the speed of obtaining results. At the same time, there are many reports in the literature about the insufficient effectiveness of existing drugs and diagnostic tests used in the practice of combating brucellosis.

Diagnostic serological reactions do not differ in the constancy of indications. Each method, even in combination with others, does not allow to identify all sick animals (2). In general, RA and RSC are not effective reactions. Each method has its own distinct advantage. The authors recommend the complex use of both reactions for laboratory diagnostics, which give positive effectiveness in the sense of complete detection of sick animals and, taking into account the reactivity of the organism, they are used repeatedly. Researchers believe that simultaneous use of diagnostic reactions complement each other, although one cannot replace the other (2,3).

EXPERIMENTAL PART

The bacteriological method is considered to be the most reliable in the diagnosis of brucellosis, with the staging of a bioassay on laboratory animals, since the isolation

of brucella cultures from pathological material is an indisputable proof of the presence of brucellosis infection. However, the negative results of this study cannot serve as an exception to the disease.(1). It should still be noted that the bacteriological method is not suitable for conducting mass lifetime studies in order to identify the source of the causative agent of infection, as it requires significant time. Therefore, the search for new, fast, specific and highly sensitive methods for the diagnosis of brucellosis is an urgent task of modern veterinary science and practice.

The achievements of modern biotechnology allow using biomolecular-genetic approaches to develop effective ways to confirm the diagnosis, one of which is the PCR method. Among the variety of hybridization methods of DNA analysis, this method is widely used in laboratory diagnostics of infectious diseases, as well as for scientific research. Many researchers note the advantage of PCR over conventional laboratory methods for diagnosing infectious diseases of animals.

The main advantages of PCR are its high 100% specificity, excessive sensitivity, which allows detecting even single living and dead cells of the pathogen, as well as DNA fragments in the minimum volume of the test sample. In addition, PCR is characterized by the universality of the pathogen detection procedure, as well as the high speed and ease of obtaining the analysis result during the working day. Moreover, PCR is effective for detecting difficult-to-cultivate, uncultivated and persistent variants of microorganisms, as well as pathogens with antigenic variability and intracellular parasites.

Polymerase chain reaction is a simple laboratory method of copying a DNA fragment using readily available reagents. As the number of copies increases exponentially, more than 100 billion copies can be obtained in just a few hours. copies.

Currently, this method is widely used both for scientific research and for diagnostics in practical healthcare, the State Sanitary and Epidemiological Surveillance Service, epizootic monitoring, in veterinary medicine for genotyping and recovery from infectious diseases.

RESULTS AND DISCUSSION

Taking into account these and other diagnostic values of PCR, we conducted comparative studies to assess the degree of intensity of the epizootic process for

brucellosis using a PCR test and generally accepted laboratory methods. The work was carried out on the basis of the Regional Veterinary Laboratory of the Turkestan region in 2019 and 2020. At the same time, over the past two years, we have examined 2460 samples of biomaterials obtained from animals with brucellosis by bacteriological and PCR methods. The results of the studies are presented in Table 1.

Table 1

Results of diagnostic examination of pathological materials of cattle and small cattle for brucellosis by bacteriological method and PCR method for 2019 and 2020 in Turkestan region

№	Years	Number of samples examin	Characteristics of the studied materials	Type animals	Highlighted positive		
					Bakter. method	PCR	Serol. method
1	2019	713	Parenchymal organs and lymph nod	KPC MPC	- -	- 6	- -
2		649	Parenchymal organs and lymph nod	KPC MPC	-	-	-
3		1 270 248	Blood serum	KPC	-	-	515
4		4 422 261	Blood serum	MPC	-	-	728
5	2020	1098	Parenchymal organs and lymph nod	KPC MPC	- -	- 8	- -
		1 335 315	Blood serum	KPC	-	-	432
7		5 058097	Blood serum	MPC	-	-	864
8	Total				-	14	2539

From the data in Table 1, it can be seen that as a result of bacteriological studies of 1362 samples in 2019, 1098 samples in 2020 of biological material from positively reacting to brucellosis in RSC and RBP, no brucella cultures were isolated, whereas as a result of PCR from a suspension of prenchymatous organs of 14 animals, a specific shining fragment of Brusella DNA was detected.

According to the results of the study, the bacteriological study failed to sow brucella in any sample of infected animals. In our opinion, this apparently depends on the quality of the culture media used for sowing, as well as on the number of localized microbial bodies in the pathological material. Consequently, it becomes expedient to use the PCR method in place of a bacteriological method that captures

not only live, but also killed microbial cells, as well as their derivatives in the material under study.

The PCR method is ahead of the bacteriological method in sensitivity due to the use of the Bru- com test system based on the application of PCR analysis, when examining pathological material from cattle from an epizootic focus for brucellosis. The results of bacteriological studies, unlike PCR, depend on the storage conditions for transporting the material to the laboratory. Whereas PCR makes it possible to detect dead microorganisms in the pathological material and even the smallest fragments of DNA left after their destruction.

Consequently, the diagnosis of brucellosis of animals using the PCR method is able to provide more prompt and accurate information to prevent further spread of the causative agent of the disease and timely conduct veterinary and sanitary measures to combat brucellosis. In addition, for two years, a planned serological study revealed 2,539 animals seropositive for brucellosis antigen in the region.

CONCLUSION

Thus, the laboratory studies have shown the high diagnostic effectiveness of the PCR method in the supervision of brucellosis infection of animals in the Turkestan region.

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