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CHARACTERISTICS OF BRUCELLA CIRCULATING ON THE TERRITORY OF THE REPUBLIC OF KAZAKHSTAN

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Abstract: as you know, one of the economically and socially significant diseases that are widespread in the territory of the Republic of Kazakhstan is brucellosis, which occupies a dominant place in the general infectious pathology of animals. *Brucellosis* is one of the most dangerous zoonotic infectious diseases for humans. The results of comparative studies on the identification of the causative agent of *brucellosis* in the Republic of Kazakhstan for several years using the bacteriological method and PCR indicate that the use of the above methods to determine the epizootological status of herds of animals in the primary diagnosis of brucellosis is impractical due to the low degree of informativeness of these tests. PCR is recommended for identification and genotyping of isolated *brucella* cultures from pathological material.

As a result of diagnostic studies of biomaterial obtained from animals from economically disadvantaged brucellosis subjects of Kazakhstan and border countries (Russia and Kyrgyzstan), the authors identified brucella cultures, which were subsequently subjected to the study of their biological and molecular genetic properties. For the identification and genotyping of isolated *brucella* cultures, researchers suggest using MLVA as the most effective method for reliably determining their genotypic characteristics.

Keywords: *brucellosis*, *brucella* cultures, bacteriology, diagnostic, PCR, genotyping.

Introduction

One of the economically and socially significant diseases that are widespread in the territory of the Republic of Kazakhstan (RK) is *brucellosis*, which occupies a dominant place in the general infectious pathology of animals. Brucellosis is one of the most dangerous zooanthroponotic infectious diseases for humans [1].

Among the measures of prevention and control of *brucellosis* in animals, timely diagnosis of infection based on the use of effective laboratory research methods is the most significant [2]. Currently, serological reactions are widely used for the diagnosis of *brucellosis* of animals, which are designed to detect specific antibodies in the blood serum of the studied animals. However, the indisputable proof of the presence of *brucellosis* infection in a particular environment is the isolation of the causative agent of the disease, which is carried out using a bacteriological method that includes techniques for identifying *brucella* to biotypes. The precise determination of the species, biovars, genotypes of brucella circulating in a certain territory of the republic is important in establishing the epizootological status of the farm and in organizing anti-*brucellosis* measures.

In many countries of the world, molecular genetic research methods are used for the detection and identification of *brucella* and laboratory confirmation of the diagnosis, in particular, polymerase chain reaction (PCR), which allows, in comparison with the bacteriological method, to determine the genus and species of isolated cultures of microorganisms in a short time (during the working day). According to various researchers, PCR can be used not only to identify the microbe, but also to analyze the genetic diversity of *brucella* collected from different regions [3, 4].

Currently, despite the presence in Kazakhstan of a significant amount of scientific research in the field of diagnosis of *brucellosis* of animals, data on genetic diversity, circulating strains of brucella, are very low [5, 6].

The purpose of these studies was to conduct a genetic analysis of *brucella* strains isolated from the body of animals that was taken from regions of our republic disadvantaged by *brucellosis*.

Materials and methods

The materials for research were the official annual veterinary reporting data of the Republican Veterinary Laboratory (RVL), pathological material from animals with *brucellosis*, received from farms with *brucellosis*, the results of their own epizootological and bacteriological studies of employees of KazSRIV LLP. Bacteriological examination of pathological material and identification of *brucella* was carried out in accordance with the differential test table proposed by FAO/WHO [7].

PCR analysis was carried out according to TU 9388-187-00494189-99, using the BRU-COM test system. To determine the species belonging of the tested *brucella* isolates in S-form, PCR was used in the classical version using the AMOS kit developed by Bricker and co-authors [8]. DNA was isolated using a set of «PureLinkGenomic DNA Kits» (Invitrogen). Multiplex PCR and capillary electrophoresis (CE) were performed using an algorithm with minor changes [9]. The sizes of VNTR fragments were identified using the GeneMapper 4.1 software. The BioNumerics7.5 software (AppliedMaths, Belgium) was used to check the size of the fragment with the MLVA database. Cluster analysis was carried out on the basis of a categorical coefficient and the method of an unweighted pair of groups using arithmetic averages (UPGMA). Standard Minimum Spanning trees (MSTS) were obtained using categorical coefficients. The results of genotyping were compared with genotypes in the MLVA data bank.

Results

At the beginning of the work, in order to consider the frequency and completeness of the detectability of the causative agent of *brucellosis* of animals in the territory of the Republic of Kazakhstan, we analyzed the available RVL data of the Ministry of Agriculture of the RK for 2014-2016. The results of the analysis of the conducted dipgnostic studies of cattle and small animals for *brucellosis* for 2014-2016 in the Republic of Kazakhstan are shown in Table 1.

Table 1 – Results of diagnostic studies to identify the causative agent of brucellosis of animals in the Republic of Kazakhstan for 2014-2016

Years	The type of animals from which the brucella culture is isolated							
	cattle				small ruminants			
	Bacteriology		PCR		Bacteriology		PCR	
	Invested samples	Culture highliged	Invested samples	Culture highliged	Invested samples	Culture highliged	Invested samples	Culture highliged
2014	4539	125	4777	206	1268	107	1498	220
2015	4850	158	4878	240	1257	86	1428	159
2016	4447	144	4444	235	2371	73	2221	145
Average for 3 years	4612	142	4699	227	1632	88	1715	174

From the data in table 1, it can be seen that in 2014-2016 in the Republic of Kazakhstan, the average rate of positive cases of bacteriological studies of patmaterial from animals for *brucellosis* for 3 years was only 142 in absolute value and 3% in relative value, PCR – 227 and 4.8%, respectively. Thus, the level of confirmation of positive results of serological studies of cattle for *brucellosis*, using the bacteriological method and PCR is very low.

A similar comparative analysis of the results of diagnostic studies of small cattle for *brucellosis* showed that an average of 1,632 samples of pathological material from this animal species were subjected to bacteriological studies annually, among which 88 samples were positive, which was 5.3%, and when studying 1715 biomathermal samples with PCR, positive results were found in 174 cases (10.1%). There is also a low degree of confirmability of positive results of serological monitoring based on the use of the bacteriological method and PCR. Analyzing the results of the conducted diagnostic studies, it can be concluded that the use of the above methods of brucella isolation from pathological material to determine the epizootological status of animal herds for brucellosis is impractical due to the low degree of informativeness of these tests.

In further studies, the identification of *brucella* isolated from biomaterial obtained from animals with brucellosis in PCR using MLVA-16 was carried out. From 9 samples of biomaterial received for research on *brucellosis* from the West Kazakhstan region, 7 cultures of *B. abortus*, 2 – *B. melitensis*, and 3 cultures of *B. melitensis* from the Zhambyl region were isolated using the bacteriological method. When studying the molecular biological characteristics based on MLVA-16 isolated *brucella* strains from the animal body, it was found that *B. melitensis* circulating among animals in the West Kazakhstan Region belongs to the third genotype, which is genetically similar to the pathogens of *brucellosis* of this species common in the Southern regions of Kazakhstan, and 7 strains of *B. abortus* isolated from the body of cattle - to the second genotype.

Analysis of the results of genotyping of *brucella* cultures circulating among animals of the West Kazakhstan region showed that the third genotype of *brucella* species *melitensis* has a wide distribution throughout the territory of the Republic of Kazakhstan. Thus, the presence of genetic uniformity of the population of *B.melitensis* in Kazakhstan suggests their origin from a common ancestor. The genotypes of abortus brucella species are unique, as they were first discovered on the territory of Kazakhstan. The observed wide distribution of the third genotype of *brucella* species melitensis throughout our country may be the result of uncontrolled livestock trade.

In order to find out the true epizootic situation of *brucellosis* of animals, scientists of KazSRIV LLP conducted their own diagnostic studies of cattle and small cattle, camels and

carnivores (dogs) within the framework of scientific research in 2018-2020. The selection of biomaterial from animals (blood sera for serological studies, whole blood, organs and lymph nodes for bacteriological studies and PCR) was carried out in various livestock farming entities of 14 regions of the republic. Economic entities for diagnostic studies for brucellosis were selected based on the analysis of available official veterinary reporting data on the state of the epizootic situation: a rural district with a high, medium degree of animal morbidity with *brucellosis*. Based on the fact that two types of *brucella* - B are of the greatest epizootological significance. abortus and *B. melitensis*, the typical hosts of which are cattle and small cattle, the selection of experimental districts and rural districts in order to study the epizootic situation was carried out according to the incidence of *brucellosis* of these two animal species, as well as camels and dogs.

For bacteriological studies and PCR, samples of pathological material were taken from aborting females, animals with clinical signs characteristic of brucellosis, as well as from animals that reacted positively to brucellosis by serological reactions, with high antibody titers.

The results of our own diagnostic studies of animals for *brucellosis* conducted in 2018-2020 are shown in Figure 1.

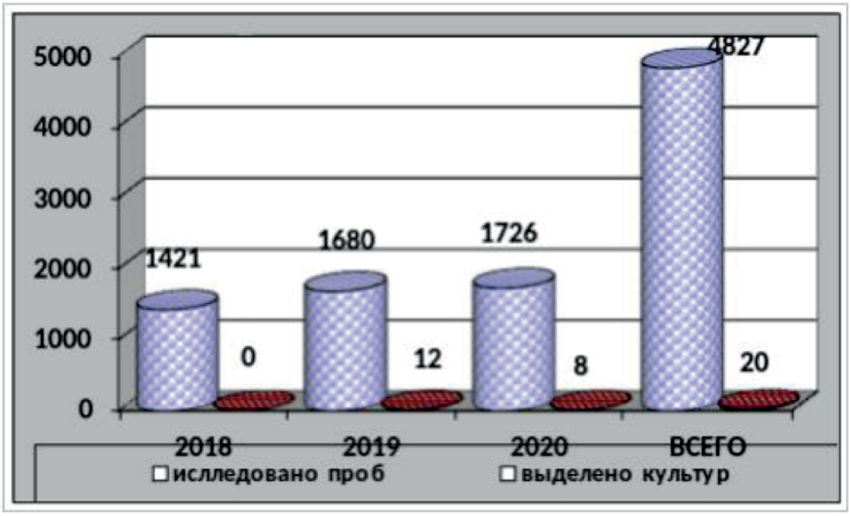

Figure 1 – Results of own animal studies on brucellosis using the bacteriological method for 2018-2020

As can be seen from Figure 1, in 2018, 1421 samples of biomaterial from cattle, small cattle, camels and dogs from various regions of the Republic of Kazakhstan were examined using the bacteriological method. At the same time, no *brucella* cultures were isolated.

In 2019, 1,680 samples of biomaterial obtained from the above 4 animal species from various regions of the Republic of Kazakhstan were examined. As a result of the diagnostic studies carried out, 12 cultures were isolated, which were subjected to genotyping.

In 2020, 1726 samples of biomaterial (pieces of parenchymal organs, lymph nodes, whole blood of cattle, small cattle, camels and dogs) from various regions of the Republic of Kazakhstan were bacteriologically examined. During this year, 8 cultures of *brucella* were isolated from the studied samples of biomaterial, including 2 cultures of the abortus species from cattle of Kostanay region, 1 – *melitensis* species from small cattle and 3 cultures of the abortus species from cattle of Aktobe region, and 2 cultures of the abortus species from cattle of East Kazakhstan region for which passports were developed indicating the studied phenotypic and genotypic properties.

In total, over three years, a total of 4827 samples of biomaterial obtained from animals responding positively to *brucellosis* were bacteriologically examined, of which 20 *brucella* cultures were isolated, including 0 in 2018, 12 in 2019 and 8 in 2020, which were subjected to the study of their phenotypic and genotypic properties with subsequent registration of passports and by depositing in the official collection of microorganisms. (the results are presented in table 2).

Table 2 – Results of the study of phenotypic and molecular genetic properties of epizootic *brucella* cultures isolated from biomaterial samples taken from animals

Name of the region	The type of animals from which the culture is isolated	Type of selected culture brucella	Genotype of the isolated brucella culture	Geography of distribution of isolated brucella cultures (in the regions of the Republic of Kazakhstan and in other countries)
Almaty	Carnivores (dog)	*B. abortus* (biovar 3)	genotype 2 (MLVA)	West KZ region, East KZ region, Almaty region; Portugal
West KZ region	cattle	*B. abortus* (biovar 3)	genotype 2 (MLVA)	West KZ, East KZ, Almaty region; Portugal
	cattle	*B.melitensis* (biovar 3)	genotype 33 (MLVA)	Almaty region; Turkey and China from people
	small cattle	*B. melitensis* (biovar 3)	genotype 33 (MLVA)	
Kyzylordin-skaya	small cattle	*B. abortus* (biovar 1)	genotype 7 (MLVA)	USA, West KZ region, Atyrau region; Portugal
Aktobe	cattle	*B. abortus* (biovar 3)	genotype 1 (MLVA)	Almaty and Akmola regions, West KZ region; Brazil
	cattle	*B. abortus* (biovar 3)	genotype 1 (MLVA)	Almaty and Akmola regions, West KZ region; Brazil
	cattle	*B. abortus* (biovar 3)	genotype 1 (MLVA)	Almaty and Akmola regions, West KZ region; Brazil
	cattle	*B. abortus* (biovar 3)	genotype 1 (MLVA)	Almaty and Akmola regions, West KZ region; Brazil
	small cattle	*B. melitensis* (biovar 1)	genotype 33 (MLVA)	Almaty region; Turkey and China from people
Kostanay	cattle	*B. abortus* (biovar 3)	genotype 1 (MLVA)	Almaty and Akmola regions, West KZ region; Brazil
	cattle	*B. abortus* (biovar 3)	genotype 1 (MLVA)	Almaty and Akmola regions, West KZ region; Brazil
East KZ region	cattle	*B. abortus* (biovar 3)	genotype 33 (MLVA)	WKR, East KZ region, Almaty region
	cattle	*B. abortus* (biovar 3)	genotype 33 (MLVA)	WKR, East KZ region, Almaty region

As the data in Table 2 show, brucella cultures were isolated from biomaterial obtained from animals from six regions of the Republic of Kazakhstan. In 2019, the isolation of brucella cultures from atypical hosts was noted: from two dogs of the Almaty region - *B. abortus* (biovar 3; genotype 2), from the cattle of the West Kazakhstan region - *B. melitensis* (biovar 3; genotype 33) and from the small cattle of the Kyzylorda region - *B. abortus* (biovar 1; genotype 7).

In 2019, there was 12 brucella cultures were isolated, including 2 *melitensis* species and 10 *abortus* species, in 2020 8 cultures, including one *B. melitensis* and 7 *B. abortus*.

Phenotypic and molecular analyses performed for *Brucella* species identified 64 *B. abortus* isolates (59.6%), 37 *B. melitensis* isolates (39.4%) and 1 B. *suis* isolate (1.0%) in the sample panel. The analysis made it possible to identify species-specific clusters for *B. abortus* and *B. melitensis*. Cluster analysis showed the presence of 31 genotypes, identifying 17 strains from 64 isolates of *B. abortus* and 12 strains from 37 isolates of *B. melitensis*. Among *B. abortus* isolates, the most common genotype was GT20, found in 13 foci located in East Kazakhstan, West Kazakhstan, Almaty and Akmola regions. GT20 has been circulating in Kazakhstan for a long time, almost 70 years (1948-2016). The same observation can be made for other genotypes of *B. abortus*, such as GT1 and GT22, which are distributed in different regions of the country. It is noteworthy that seven genotypes of *B. abortus* (GT6, GT10, GT22, GT25, GT26, GT29 and GT31) were new, no records were found in the international MLVA database.

Among *B. melitensis* isolates, the most common genotype is GT3, detected during outbreaks of brucellosis in the Almaty region (2011), WKR (2015 and 2017) and EKR (2017). This genotype was identified in cattle and small cattle GT3 was previously identified in a sample of material from a Russian flock of sheep in 1953.

Another common genotype is GT18, which was limited to the Almaty region, which was not previously reported in the MLVA database; similarly, four more genotypes of *B. melitensis* (GT5, GT9, GT14 and GT15) were discovered for the first time. The MLVA-15 genotyping phylogeography was used to evaluate the phylogeographic relationships of samples with those deposited in the MLVA database.

All Kazakh and Russian isolates are combined into the group «*Abortus C*». The analysis showed that almost half of the *B. abortus* strains are distributed in three clusters.There are also a couple of clusters in which Kazakh isolates have the same profile with Italian, French and Chinese strains. In addition, the MST analysis showed 8 clusters, including genotypes that occur exclusively in Kazakhstan; two of these clusters were represented by single strains. The phylogeographic patterns of 37 *B. melitensis* isolates were compared with MLVA profiles from the database. All Kazakh, Russian and Kyrgyz isolates were classified into the «Eastern Mediterranean» group. Thirteen Kazakh and one Russian isolates form a cluster with previous Kazakh isolates and strains from China. This cluster of B. melitensis includes a strain identified in Turkey in 2017. The other two clusters demonstrate a genetic correlation between strains from Kazakhstan and China. Some of these Chinese strains have been isolated from sick people. Three clusters included genotypes found exclusively in Kazakhstan: one cluster was represented by a 1970 strain, while the other two were represented by isolates that were limited in the Almaty region. One cluster included a Russian isolate from humans, a strain from Kyrgyzstan and a Chinese strain from humans (2015). One field isolate of *B. melitensis* from Russia was included in the Kazakh-Chinese cluster, and the other formed a cluster of one strain associated with strains from Kazakhstan and China.

MLVA-15 was used to determine small-scale epidemiological relationships in Kazakhstan. The first clade included six strains of *B. abortus,* GT2, isolated during 2015 from cattle from three settlements of the West Kazakhstan Region (Merey, Zhangala, Kushumsky) belonging to different districts (Taskalinsky, Zhangalinsky, Zelenovsky). Epidemiological investigation showed that the villages of Kushumsky and Merey border each other and the grazing of animals is carried out on the same pasture. The village of Zhangala is far away, in an area with a high prevalence of brucellosis. When in 2010-2014 the population from the village of Zhangaly began to move to the Taskalinsky and Zelenovsky districts, GT2 spread as a result of the migration flow. The second clade included strains of *B. abortus*, GT22. Four archival isolates from Almaty region belonged to this genotype (1960-1968) together with three samples from East Kazakhstan region in 2016. The latter came from three villages: Ust-Kamenogorsk, Bozanbai and Ablaketsky, located in the Ulan district. Classical epidemiology

confirmed the results of molecular epidemiology: the villages of Bozanbai and Ablaketsky are located next door, the animals graze on the pasture «Sandyktas». The village of Ust-Kamenogorsk is located at a distance of 70 km, the animals graze on the «Kyzyl-su» pasture. However, commercial animal flows are reported among the farms of these villages.

The genotypes of *melitensis* brucella isolated in Russia and Kyrgyzstan showed a complete correlation with those of Kazakh and Chinese strains, which indicates the preservation of the common genotype in the Eurasian region. At the same time, the spread of 7 new genotypes of *B. abortus* and five of B. melitensis in the Republic of Kazakhstan was noted. Some outbreaks were characterized by multiple MLVA-15 genotypes.

Brucellosis, registered in 2015 among animals in the city of Tekeli, Almaty region, was caused by *B. melitensis* genotypes GT4 and GT5. Brucellosis pathogens *B. abortus* GT20 and *B. melitensis* GT18 were circulating in the village of Zholaman in 2016. Based on the results obtained, it can be stated that uncontrolled migration of livestock and weak measures to create and preserve biosafety are the root cause of the emergence and spread of *brucellosis*.

Discussion

At the initial diagnosis of *brucellosis* of animals in previously prosperous farms, according to the Veterinary and Sanitary Rules (Order of the Ministry of Agriculture of the Republic of Kazakhstan dated June 29, 2015 No. 7-1/587), animals that have shown positive results in serological studies for *brucellosis* or have clinical signs similar to *brucellosis* are subject to bacteriological examination and PCR examination for *brucellosis*. Upon receipt of positive results of these research methods, the diagnosis and status of animal herds for *brucellosis* is considered established and restrictions are imposed on farms and recreational activities are carried out. If the results of these studies are negative, it is necessary to continue repeated serological studies of animals to confirm the diagnosis. Analyzing the results of the conducted diagnostic studies, it can be concluded that the use of the above methods of *brucella* isolation from pathological material to determine the epizootological status of animal herds for *brucellosis* is impractical due to the low degree of informativeness of these tests.

But the cases of detection of the causative agent of *brucellosis* in the studied biomaterials not only confirms the presence of brucellosis infection in the herd, but also serves as a scientific justification to change the tactics of health measures, for example, in such cases, it is recommended that the animals of the entire herd be slaughtered.

The analysis of official data provided by the RVL of the Ministry of Agriculture of the Republic of Kazakhstan on the results of diagnostic studies of animals for *brucellosis* in the Republic of Kazakhstan and the results of own research of KazSRIV employees was carried out. It was found that the degree of confirmability of positive results of serological methods of animal biomaterial studies for *brucellosis* using the bacteriological method (or PCR) is quite low, which does not allow us to recommend the last two tests to determine the status of animal herds for brucellosis.

The applicants used MLVA for genotyping a panel of 102 *brucella* isolates isolated from 1935 to 2017 from patmaterial obtained from humans and animals from 8 regions of Kazakhstan and border countries (Russia, Kyrgyzstan). The results of phylogeography based on MLVA-15 showed that the strains of *B. abortus* and *B. melitensis* belong to the lines «*Abortus C*» and «Eastern Mediterranean», respectively. It has been established that *B. abortus* strains circulating in the territories of Kazakhstan and Russia are genetically related to Portuguese, Brazilian and American isolates.

It was found that most of the Kazakh isolates of *B. melitensis* are associated with Chinese strains. In a small-scale analysis based on MLVA-15, 17 genotypes of *B. abortus* and 12 *B. melitensis* were identified, among which 12 are new, previously unknown. Epizootological

information previously obtained using well-known classical techniques can be supported by established new molecular information for two clusters of group *B. abortus*, which indicates the possibility of using MLVA as a modern informative tool for determining the breadth of the distribution area of brucellosis pathogens in the territories of the Republic of Kazakhstan and neighboring countries and the possible interchange between these countries with brucellosis infection.

The research results show that molecular genotyping can be used to identify circulating varieties of *brucella* strains on the territory of the republic, the results of which may be important for the effective scientifically-based organization of anti-brucellosis measures in Kazakhstan.

Conclusion

Due to the fact that brucella are slow-growing microorganisms and it is bacteriologically possible to detect *brucella* only after 3 to 5 weeks, the molecular biological method - PCR, is an operational method for detecting the causative agent of *brucellosis* and timely in diagnosis, easy to carry out, not inferior in effectiveness to the bacteriological method.

A comparative study of the results of the bacteriological method and PCR in the study of biomaterial obtained from animals with positive analyses of preliminary serological tests showed the inexpediency of using these methods to determine the status of herds of animals during the initial diagnosis of *brucellosis* and further choice of tactics for anti-*brucellosis* measures.

The MLVA method is recommended for identification and genotyping of isolated *brucella* cultures. The molecular genetic characteristics of *brucella* established with the help of MLVA will prove to be useful information during epizootological analysis, which can be used to track the sources of infection of animals and humans in previously prosperous regions of the republic.

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ҚАЗАҚСТАН РЕСПУБЛИКАСЫ АУМАҒЫНДА ТАРАЛҒАН БРУЦЕЛЛАЛАРДЫҢ СИПАТТАМАСЫ

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Аннотация: Қазақстан Республикасының (ҚР) аумағында кең таралған экономикалық және әлеуметтік маңызды аурулардың бірі жануарлардың жалпы жұқпалы патологиясында басым орын алатын бруцеллез екені белгілі. Бруцеллез – адам үшін аса қауіпті

зоонозды жұқпалы аурулардың бірі болып табылады. Қазақстан Республикасында бруцеллез қоздырушысын анықтауда бірнеше жылдар бойы ПТР және бактериологиялық әдісті қолдана отырып жүргізілген салыстырмалы зерттеулердің нәтижесінде бруцеллезге алғашқы балау арқылы мал табындарының эпизоотологиялық жағдайын анықтау үшін жоғарыда аталған әдістер өздерінің беретін ақпаратының төмен болуына байланысты қолдану тиімсіз. ПТР патологиялық материалдан бөлініп алынған бруцеллалардың түрін ажырату және генотиптендіру үшін қолдану ұсынылады.

Қазақстанның және шекаралас елдердің (Ресей мен Қырғызстан) бруцеллезден сау емес шаруашылықтарынын алынған биоматериалды диагностикалық зерттеулердің нәтижесінде авторлар бруцеллез өсінділерін бөліп алып, олардың биологиялық және молекулалық-генетикалық қасиеттерін анықтады. Бөліп алынған бруцелла өсінінің түрін анықтау және генотиптендіру үшін зерттеушілер олардың генотиптік сипаттамаларын дәл анықтауға мүмкіндік беретін тиімді әдіс ретінде MLVA қолдануды ұсынады.

Түйін сөздер: бруцеллез, бруцелла өсіндісі, бактериология, балау, ПТР, генотиптендіру.

ХАРАКТЕРИСТИКА БРУЦЕЛЛ, ЦИРКУЛИРУЮЩИХ НА ТЕРРИТОРИИ РЕСПУБЛИКИ КАЗАХСТАН

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Аннотация: как известно, одним из экономически и социально значимых заболеваний, широко распространённых на территории Республики Казахстан (РК) является бруцеллёз, который занимает главенствующее место в общей инфекционной патологии животных. Бруцеллез является одной из наиболее опасных для людей зооантропонозных инфекционных болезней. Результаты сравнительных исследований по индикации возбудителя бруцеллеза в РК за несколько лет с помощью бактериологического метода и ПЦР свидетельствуют, что использование вышеуказанных методов для определения эпизоотологического статуса стад животных при первичной постановке диагноза на бруцеллез является нецелесообразным из-за низкой степени информативности этих тестов. ПЦР рекомендуется для идентификации и генотипирования выделенных культур бруцелл из патологического материала.

В результате проведенных диагностических исследований биоматериала, полученного от животных из неблагополучных по бруцеллезу хозяйствующих субъектов Казахстана и приграничных стран (России и Киргизстана), авторами выделены культуры бруцелл, которые в последующем были подвержены изучению их биологических и молекулярно-генетических свойств. Для идентификации и генотипирования выделенных культур бруцелл исследователли предлагают использовать MLVA, как наиболее эффективный метод, позволяющий достоверно определять их генотипические характеристики.

Ключевые слова: бруцеллез, культуры бруцелл, бактериология, диагностика, ПЦР, генотипирование.