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СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ПРИМЕНЕНИЯ ИФА С МОЛОКОМ И СЫВОРОТКОЙ КРОВИ ДЛЯ ДИАГНОСТИКИ БРУЦЕЛЛЕЗА КРУПНОГО РОГАТОГО СКОТА

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Иммуноферментный анализ как более чувствительный метод позволяет выявить специфические антитела в пробах сборного молока. С учетом доступности и простоты выполнения данного метода исследование методом ИФА сборного молока в хозяйствах может стать важнейшим элементом в системе противобруцеллезных мероприятий по части контроля за эпизоотическим состоянием в хозяйствах. Для скрининговой экспресс-диагностики бруцеллеза крупного рогатого скота разработана методика постановки иммуноферментного анализа с молоком коров. Иммуноферментный анализ с сывороткой молока является специфичным, чувствительным, простым в постановке, учете и интерпретации результатов методом. Установлено, что условия хранения и транспортировки проб молока, соответствующие значениям комнатной температуры и приводящие к сквашиванию, не оказывают влияния на уровень специфических противобруцеллезных иммуноглобулинов в течение 8 сут, что снимает вопрос о применении холодовой цепи при транспортировке до места проведения анализа проб молока, подлежащих исследованию. При отработке оптимальной пробоподготовки разница в специфическом сигнале при постановке ИФА между сывороткой молока, полученной при высокоскоростном центрифугировании, и сывороткой молока, полученной методом сквашивания в течение 24 ч, составляла менее 10%. В связи с этим для подготовки проб сыворотки молока при исследовании ИФА выбрано скоростное центрифугирование. Изучены возможности применения ИФА с сывороткой молока на вакцинированном и не вакцинированном против бруцеллеза поголовье крупного рогатого скота. Молоко и кровь для исследования в ИФА необходимо брать через 6 мес и более после вакцинации (в инструктивные сроки). Установлен высокий уровень корреляции между данными ИФА с молоком и данными ИФА с сывороткой крови вне зависимости от эпизоотического или иммунного статуса (благополучные и неблагополучные стада, привитые и непривитые животные), который составил 86,8–92,0%.

Ключевые слова: бруцеллез, крупный рогатый скот, поствакцинальная диагностика, сыворотка крови, молоко, иммуноферментный анализ

COMPARATIVE STUDY OF THE ELISA USE WITH MILK AND BLOOD SERUM FOR BOVINE BRUCELLOSIS DIAGNOSIS

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Enzyme immunoassay, being a more sensitive method, makes it possible to identify specific antibodies in samples of combined milk. The ELISA study of harvested milk in farms can become an

important element in the system of anti-brucellosis measures regarding the control of the epizootic state in farms taking into account the availability and ease of implementation of this method. For screening express-diagnostics of bovine brucellosis the method of enzyme immunoassay with milk of cows has been developed. ELISA with milk serum is specific, sensitive, easy to formulate, account for and interpret the results. It has been found that the conditions of storage and transportation of milk samples corresponding to room temperature values and leading to fermentation do not affect the level of specific anti-viral immunoglobulins for eight days, and the question of the use of a cold chain during transportation of milk samples to be examined to the place of analysis can be withdrawn from the agenda. When working out the optimal sample preparation, the difference in the specific signal when setting the ELISA between the milk serum obtained by high-speed centrifugation and the milk serum obtained by fermentation for 24 hours was less than 10%. Therefore, high-speed centrifugation was chosen for the preparation of milk serum samples during the ELISA study. The possibilities of using ELISA with milk serum on vaccinated and non-vaccinated cattle against brucellosis have been studied. Milk and blood for testing in the ELISA should be taken 6 months or more after vaccination (within the instructional time frame). A high level of correlation was established between ELISA data with milk and ELISA data with blood serum, regardless of epizootic or immune status (satisfactory and unfavorable herds, vaccinated and unvaccinated animals), which amounted to 86.8–92.0%.

Keywords: brucellosis, cattle, post-vaccination diagnostics, blood serum, milk, enzyme immunoassay

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INTRODUCTION

Brucellosis in agricultural animals still prevails in our country. There is still a risk of human infection, making the problem of completely eradicating this infection relevant.

Multiple vaccinations pose a challenge for post-vaccination diagnosis. Therefore, a complex of tests is used for brucellosis diagnosis, including agglutination reaction (AR), complement fixation test (CFT), indirect hemagglutination reaction (IHA), immunodiffusion reaction (IDR) with O-PS antigen, enzyme-linked immunosorbent assay (ELISA), and others. The subject of research is blood serum [1–3]. It is important to find simple, easily accessible express methods for monitoring the well-being of farms regarding brucellosis [4]. Tests using milk can be one such method[1]. Studying milk using the ring test (RT) and IHA allows for a simple sample collection method for brucellosis diagnosis, which does not cause stress to animals. It also involves the examination of the mammary gland, which is one of the sites of entry, localization, and spread of the infection in brucellosis [5, 6].

The most promising research methods are the ring test and enzyme-linked immunosorbent assay with milk, which have received high praise from domestic and foreign researchers [7–9]. The ring test is the most widely used method for preliminary examination of brucellosis in cattle herds and individual animals. Titre fluctuations in individual udder quarters indicate brucellosis infection, while identical titres in all udder quarters are more likely post-vaccination titres. The

[1]*Popova T.G.* Diagnostic value of research methods for brucellosis of milk of cows reimmunized with brucellosis vaccines (strain 19 and strain 82): Extended abstract of candidate's thesis in Veterinary Medicine, Novosibirsk, 1990, 16 p.

FAO/WHO Expert Committee on Brucellosis recommends this reaction for widespread use in all countries as an additional method [5, 6, 10].

ELISA can be used for mass screening of cattle herds and for making a final diagnosis in individual animals[2]. Compared to other methods for detecting antigens and antibodies, enzyme-linked immunosorbent assays have several advantages: high sensitivity, specificity, reproducibility of results, stability when storing all necessary reagents (up to a year or more), ease of conducting the reaction, and the ability to use minimal volumes of the material being tested, as well as instrumental (qualitative and quantitative) recording of the reaction and the possibility of automating all its stages [11, 12]. The material for examination by the enzyme-linked immunosorbent assay can be udder secretion from dry cows, colostrum, and milk[3]. ELISA with milk is conducted in the same way as with blood serum [13].

It has already been proven that the use of ELISA for diagnosing brucellosis in agricultural animals is quite effective. In a comparative study of cows using this test, antibodies were detected in blood serum 50–72% more than in AR, CFT, AR with rivanol, and RBT (Rose Bengal Test) combined, and in milk 5–6 times more than in the ring test [8, 13].

ELISA can determine antibody titres, which is especially important when examining vaccinated animals, as it is necessary to distinguish post-vaccination reactions from those occurring during natural brucellosis [11, 14, 15].

Enzyme-linked immunosorbent assay is a more sensitive method, allowing the detection of specific antibodies in bulk milk samples. Considering the accessibility and simplicity of this method, research using ELISA with bulk milk on farms can become a crucial element in the brucellosis control system regarding the epidemiological status in farms [11, 14].

The purpose of the study is the comparative examination of the developed ELISA test with milk and with blood serum in brucellosis of cattle.

MATERIAL AND METHODS

The studies were conducted in the laboratory of optimization of anti-epizootic systems of the Institute of Experimental Veterinary Science of Siberia and the Far East of the SFSCA RAS.

To develop a methodology for obtaining milk serum for setting up milk-based enzyme-linked immunosorbent assay (ELISA) for the diagnosis of brucellosis in cattle, milk samples were collected from vaccinated and unvaccinated cows. Blood samples were simultaneously collected from the same animals for comparative analysis.

Milk serum was obtained from milk through high-speed centrifugation and clotting, which was used for the ELISA. Blood serum was obtained using traditional methods. ELISA with milk serum and blood serum was carried out in the classical manner. The reaction results were recorded using a spectrophotometer, and optical density was measured at a wavelength of 450 nm.

In parallel with the serum milk and blood serum research, a mandatory complex of serological tests for brucellosis diagnosis was conducted, including agglutination reaction, complement fixation reaction with S antigen, and immunodiffusion reaction with O-PS antigen.

The epizootological analysis of bovine brucellosis was carried out on the basis of statistical data, materials of veterinary records and reports, results of laboratory studies conducted in veterinary laboratories, and literature data.

RESULTS AND DISCUSSION

During the optimization of sample preparation, it was found that the difference in specific signal in the ELISA between milk serum obtained through high-speed centrifugation and milk serum obtained by clotting for 24 hours was less than 10%, which is not essential for analysis (see Table 1).

[2]*Verkovsky O.A.* Laboratory diagnostics of infectious diseases of cattle using immunoenzyme analysis (leukosis, foot-and-mouth disease, brucellosis) // Veterinaria Kubani, 2007, N 2, pp. 11-12.

[3]*Vanzini V.R., Aguirre N., Lugaresi C.I., Echaide S.T., Canavesio V.G., Guglielmone A.A., Marchesino M.D., Nielsen K.* Evaluation of an indirect ELISA for the diagnosis of bovine brucellosis in milk and serum samples in dairy cattle in Argentina // Preventive veterinary medicine, 1998, N 3, pp. 211–217. DOI: 10.1016/s0167-5877(98)00080-4.

Табл. 1. Уровень сигнала в ИФА специфических противобруцеллезных иммуноглобулинов молока при различных типах пробоподготовки

Table 1. The signal level in the ELISA of specific anti-brucellosis immunoglobulins of milk in various types of sample preparation

Sample number	Sample type	High-speed centrifugation, $D_{450, O.E.}$	Fermentation, $D_{450, O.E.}$
1	Negative	0,154	0,168
2	»	0,122	0,136
3	»	0,126	0,132
4	»	0,137	0,144
5	Positive	1,857	1,890
6	»	0,560	0,578
7	»	0,823	0,843
8	»	0,455	0,623
9	»	2,216	2,237
10	»	0,445	0,534
11	»	1,235	1,362
12	»	1,583	1,603
13	»	1,129	1,341
14	»	1,529	1,627
15	»	0,481	0,503
16	»	0,639	0,681
17	»	0,885	0,912
18	»	2,251	2,282
19	»	0,772	0,781
20	»	0,981	0,923
21	»	1,112	1,182
22	»	0,991	1,121
23	»	1,651	1,668
24	»	1,225	1,284

Research on the duration of the preservation of specific immunoglobulins in milk serum during transportation and storage at room temperature was conducted. It was established that the level of the specific signal in positive milk serum in the ELISA remained unchanged for 7–8 days, after which a decrease was observed, leading to non-reactivity in low-titer samples (see Table 2).

Research was also conducted to study the correlation between ELISA with milk and ELISA with blood serum. Previously, it was established that ELISA is a specific and sensitive method for diagnosing brucellosis in cattle. The results of the mandatory complex of serological tests were also taken for comparison.

In the first group of animals from a disease-free farm, 50 cows that had been repeatedly vaccinated against brucellosis were selected. Samples of milk and blood were simultaneously taken from them shortly after the latest revaccination (1.5 months after).

In the analysis of vaccinated animals using milk-based ELISA, positive results were obtained in 46 samples (92%), while in ELISA with blood serum, positive results were obtained in all 50 samples (100%). Only in four milk samples, the results were questionable, with positive results in the blood serum samples, resulting in a correlation of 92.0% of the examined samples (see Table 3).

When comparing the results obtained with traditional blood serum tests, it was found that only 16 animals showed low titers in the agglutination reaction (AR), which is characteristic of post-vaccination reactions. The results of complement fixation tests with S antigen and immunodiffusion with O-PS antigen were negative.

This group of animals was vaccinated against brucellosis, and blood and milk were collected from them at early non-standard intervals. Therefore, the positive results in both milk-based ELISA and blood serum ELISA can be attributed to the higher sensitivity of the method. It also becomes evident that milk and blood from vaccinated animals for immunoenzyme analysis should be collected after 6 months or more following vaccination.

39 cows were selected in the second group of animals, and samples of milk and blood were

Табл. 2. Уровень сигнала в ИФА специфических противобруцеллезных иммуноглобулинов молока в различные сроки инкубации

Table. 2. Signal level in the ELISA of specific anti-brucellosis immunoglobulins of milk at different incubation periods

Incubation, days	Sample number											
	1	2	3	4	5	6	7	8	9	10	11	12
1	1,857	0,560	0,823	0,455	2,216	0,445	1,235	1,583	1,129	1,529	0,481	0,639
2	1,861	0,564	0,818	0,455	2,231	0,443	1,238	1,579	1,220	1,534	0,501	0,649
3	1,868	0,568	0,842	0,458	2,239	0,454	1,309	1,603	1,258	1,561	0,534	0,651
4	1,867	0,561	0,839	0,463	2,225	0,461	1,287	1,591	1,129	1,553	0,498	0,648
5	1,849	0,571	0,835	0,458	2,258	0,449	1,277	1,599	1,209	1,549	0,511	0,643
6	1,872	0,567	0,846	0,465	2,256	0,462	1,289	1,584	1,131	1,551	0,489	0,650
7	1,864	0,564	0,829	0,467	2,273	0,458	1,301	1,595	1,142	1,521	0,490	0,649
8	1,853	0,569	0,801	0,461	2,254	0,449	1,278	1,601	1,132	1,499	0,488	0,647
9	1,812	0,559	0,811	0,435	2,234	0,438	1,263	1,584	1,113	1,541	0,485	0,635
10	1,801	0,551	0,802	0,448	2,012	0,421	1,235	1,579	1,108	1,488	0,451	0,621
11	1,785	0,502	0,783	0,425	1,983	0,398	1,176	1,521	0,961	1,112	0,455	0,599
12	1,631	0,463	0,731	0,385	1,832	**0,335**	0,890	1,381	0,633	0,899	0,401	0,457
13	1,402	0,364	0,632	**0,322**	1,788	**0,301**	0,670	1,278	0,563	0,654	0,378	0,384
14	1,299	**0,320**	0,554	**0,301**	1,701	**0,256**	0,598	1,012	0,488	0,599	**0,322**	**0,341**

Note. Samples with an ELISA result as unreactive are marked in bold type.

taken simultaneously. This group of animals had never been vaccinated against brucellosis. As the research showed, a significant number of diseased animals were detected in the herd, which was considered unfavorable for brucellosis.

In the unvaccinated population of cattle, positive results were obtained in 34 out of 39 milk samples (87.2%) in milk-based ELISA and in 37 out of 38 blood serum samples (97.2%) in ELISA with blood serum. A correlation of 86.8% was registered in 33 out of 39 examined samples (see Table 4).

Analyzing the results obtained, it can be concluded that regardless of the epizootic or immune status (disease-free and unfavorable herds, vaccinated and unvaccinated animals), the correlation between milk-based ELISA and blood serum ELISA ranges from 86.8% to 92.0%.

CONCLUSIONS

1. It has been established that the enzyme-linked immunosorbent assay (ELISA) diagnostic test system developed by the Institute of Experimental Veterinary Science of Siberia and the Far East (SFECA RAS) in collaboration with OOO RPC Sibbiotest is suitable for determining specific anti-brucellosis immunoglobulins in milk serum.

2. It is shown that storage and transportation conditions of milk samples that result in clotting

Табл. 3. Результаты исследования молока и сыворотки крови на вакцинированном против бруцеллеза поголовье крупного рогатого скота (благополучное стадо)

Table 3. Results of milk and blood serum testing on brucellosis-vaccinated cattle (healthy herd)

Item No.	Inventory No.	ELISA with milk		ELISA with blood serum		Other serologic tests		
		Indicator	Result	Indicator	Result	AR	CBR-S	IDR
1	KZF192340333	2,272	Positive	1,861	Positive	Negative	Negative	Negative
2	KZF191261907	1,744	»	2,011	The same	»	The same	The same
3	KZF192340331	2,232	»	2,288	»	50 ME*	»	»
4	KZF192340343	1,565	»	1,960	»	Negative	»	»
5	KZF192340386	2,302	»	1,561	»	50 ME*	»	»
6	KZF192340334	1,852	»	2,080	»	Negative	»	»
7	KZF192359717	1,828	»	2,148	»	»	»	»
8	KZF192037298	0,830	»	2,210	»	»	»	»
9	KZF192340335	1,527	»	2,040	»	50 ME*	»	»
10	KZF192340328	1,017	»	2,088	»	Negative	»	»
11	KZF192359715	1,619	»	1,913	»	50 ME*	»	»
12	KZF192340382	0,897	»	1,419	»	Negative	»	»
13	KZF191088986	1,428	»	1,875	»	»	»	»
14	KZF191088635	2,113	»	2,424	»	50 ME*	»	»
15	KZF191088978	0,770	»	1,866	»	50 ME*	»	»
16	KZF192340348	1,290	»	1,415	»	Negative	»	»
17	KZF189138202	1,393	»	2,036	»	»	»	»
18	KZF192359721	2,283	»	2,016	»	»	»	»
19	KZF192340395	1,604	»	2,294	»	»	»	»
20	KZF191261835	2,296	»	1,898	»	»	»	»
21	KZF191089174	1,342	»	1,874	»	»	»	»
22	KZF191261922	1,016	»	2,108	»	»	»	»
23	KZF190473568	2,191	»	2,181	»	50 ME*	»	»
24	KZF192340332	0,540	Doubtful	1,558	«	Negative	«	«
25	KZF189857819	1,196	Positive	1,771	«	50 ME*	«	«
26	KZF192340392	0,906	»	1,647	»	50 ME*	»	»
27	KZF189211198	1,959	»	2,191	»	Negative	»	»
28	KZF191089171	1,898	»	2,023	»	50 ME*	»	»
29	KZF191089163	1,001	»	1,536	»	50 ME*	»	»
30	KZF189857842	0,946	»	2,126	»	50 ME*	»	»
31	KZF190096431	1,729	»	1,935	»	50 ME*	»	»
32	KZF190121536	2,113	»	2,086	»	Negative	»	»
33	KZF191261920	1,955	»	2,364	»	50 ME*	»	»
34	KZF192340369	1,897	»	1,931	»	Negative	»	»
35	KZF192340387	1,367	»	1,951	»	»	»	»
36	KZF192359725	0,843	»	1,502	»	»	»	»
37	KZF191089025	1,615	»	1,900	»	50 ME*	»	»
38	KZF191126039	1,529	»	2,089	»	Negative	»	»
39	KZF192367987	1,908	»	2,258	»	»	»	»
40	KZF192340381	0,923	»	1,112	»	»	»	»
41	KZF189429652	0,648	Doubtful	1,969	»	»	»	»
42	KZF192340337	2,074	Positive	2,223	»	»	»	»
43	KZF190096491	1,161	»	1,512	»	»	»	»
44	KZF191261908	0,373	Doubtful	1,409	»	»	»	»
45	KZF191261840	1,975	Positive	1,914	»	»	»	»
46	KZF190096500	1,084	»	2,264	»	»	»	»
47	KZF189429656	0,317	Doubtful	1,525	»	»	»	»
48	KZF190096419	0,720	Positive	2,043	»	»	»	»
49	KZF192340336	0,987	»	1,499	»	50 ME*	»	»
50	KZF192368020	2,212	»	1,967	No serum	Negative	»	»

* The result of AR 50 IU on brucellosis vaccinated cattle (in a healthy herd) is considered doubtful.

Табл. 4. Результаты исследования молока и сыворотки крови на не вакцинированном против бруцеллеза поголовье крупного рогатого скота (неблагополучное стадо)

Table 4. Results of milk and blood serum testing on bovine cattle not vaccinated against brucellosis (unfavorable herd)

Sample number	Inventory No.	ELISA with milk		ELISA with blood serum		Other serologic tests		
		Indicator	Result	Indicator	Result	AR	CBR-S	IDR
1	90097334**	2,139	Positive	2,275	Positive	Negative	1 : 10+++	Negative
2	90097053**	2,292	The same	2,321	The same	200 ME*	1 : 20++++	+48 ч
3	90633070**	2,193	»	2,317	»	Negative	1 : 5+++	Negative
4	90097363**	2,274	»	2,357	»	200 ME*	1 : 20++++	+24 ч
5	90097050**	2,146	»	2,322	»	200 ME*	1 : 20++++	+24 ч
6	90097064**	2,165	»	2,107	»	50 ME	1 : 5+++	Negative
7	90097056**	2,132	»	2,151	»	200 ME*	1 : 10++	»
8	90321543	0,142	Negative	1,208	»	Negative	Negative	»
9	90097357**	2,235	Positive	2,112	»	50 ME	1 : 20++++	»
10	90097337**	2,310	The same	2,401	»	200 ME*	1 : 20++	+48 ч
11	90097095**	2,100	»	2,276	»	Negative	1 : 20++++	Negative
12	90097324**	2,293	»	2,311	»	50 ME	1 : 5++++	«
13	90097078**	2,369	»	2,391	»	200 ME **	1 : 20++++	+24 ч
14	90097089**	0,458	Doubtful	1,553	»	50 ME	1 : 20++++	Negative
15	90097079**	2,364	Positive	2,382	»	200 ME*	1 : 20++++	+24 ч
16	90324551	0,072	Negative	0,241	Negative	Negative	Negative	Negative
17	90097371**	2,214	Positive	2,361	Positive	100 ME*	1 : 20++++	+24 ч
18	91073268**	0,018	Negative	2,252	The same	50 ME*	1 : 10++++	+48 ч
19	90097372	1,658	Positive	2,151	»	Negative	Negative	Negative
20	90097039**	2,098	The same	2,344	»	100 ME*	1 : 20+++	»
21	90097355**	2,417	»	2,334	»	200 ME *	1 : 20++++	»
22	90097336**	2,172	»	2,352	»	200 ME*	1 : 20++++	+24 ч
23	90097076**	2,390	»	2,453	»	200 ME*	1 : 20++++	+24 ч
24	90633071**	2,178	»	2,372	»	200 ME*	1 : 20+++	+24 ч
25	90097331**	2,035	»	2,450	»	50 ME	1 : 20+++	+24 ч
26	90097342**	2,207	»	2,208	»	50 ME	1 : 5++++	Negative
27	90097333**	2,259	»	2,405	»	100 ME*	1 : 20++++	+24 ч
28	90097062**	2,285	»	2,322	»	100 ME**	1 : 20+++	+48 ч
29	90097325**	2,232	Positive	2,358	Positive	50 ME	1 : 20++++	Negative
30	90321801**	2,302	The same	2,361	The same	50 ME	1 : 5++++	The same
31	90097055**	2,167	»	2,156	»	100 ME*	1 : 10++	»
32	90097362**	2,291	»	2,301	»	50 ME	1 : 20+++	»
33	90321768**	1,903	»	2,325	»	100 ME*	1 : 10+++	+48 ч
34	90097351**	2,203	»	2,260	»	200 ME*	1 : 20+++	+24 ч
35	90097356	0,670	Doubtful	1,189	»	Negative	Negative	Negative
36	90097048**	2,102	Positive	2,325	»	50 ME	1 : 10++	То же
37	97073220**	2,207	The same	2,421	»	100 ME*	1 : 20++++	+24 ч
38	91073265**	2,238	»	2,322	»	100 ME*	1:20++++	+48 ч
39	89202667**	2,330	»	2,347	Not studied	Negative	1 : 10+++	Negative

* The result of AR 100 IU and above and/or CBR 1 : 5 and above on unvaccinated against brucellosis cattle (in an unfavourable herd) is considered positive..

** The animals were recognized as sick and sent to slaughter.

at room temperature do not affect the level of specific anti-brucellosis immunoglobulins for at least 8 days. This eliminates the need for a cold chain during transportation of the milk samples to the testing site.

3. It has been found that in a vaccinated population of cattle against brucellosis, the correlation between milk-based ELISA and blood serum ELISA is 92.0%. It should be noted that milk and blood for ELISA analysis should be collected after 6 months or more following vaccination (according to the instructions).

4. In an unvaccinated population of cattle against brucellosis, the correlation between milk-based ELISA and blood serum ELISA is 86.8%, with results matching in 33 out of 39 samples.

5. Regardless of the epizootic or immune status (disease-free and unfavorable herds, vaccinated and unvaccinated animals), the correlation between milk-based ELISA and blood serum ELISA ranges from 86.8% to 92.0%.

6. The high correlation percentage provides grounds for using milk initially for brucellosis diagnosis, and if positive results are obtained, then blood can be collected from such animals for comprehensive brucellosis testing.

СПИСОК ЛИТЕРАТУРЫ

1. *Аракелян П.К., Христенко Н.В., Гайворонская Ю.Е., Димова А.С., Димов С.К., Янченко Т.А.* Технологичность разных схем иммунизации крупного рогатого скота против бруцеллеза с возможностью ранней поствакцинальной диагностики // Ветеринария. 2023. № 5. С. 11–16. DOI: 10.30896/0042-4846.2023.26.5.11-15.
2. *Аракелян П.К., Гайворонская Ю.Е., Руденко А.В., Ильин Е.Н., Димова А.С., Димов С.К., Янченко Т.А.* Серологическая реактивность здоровых овец, иммунизированных против бруцеллеза живой вакциной из штамма *B. abortus* 19 // Ветеринария. 2022. № 4. С. 21–25. DOI: 10.30896/0042-4846.2022.25.4.21-25.
3. *Гордиенко Л.Н., Новиков А.Н., Куликова Е.В.* Эффективность дифференциального теста при диагностике бруцеллеза северных оленей // Ветеринария. 2020. № 11. С. 7–10. DOI: 10.30896/0042-4846.2020.23.11.07-10.
4. *Аракелян П.К., Христенко Н.В., Гайворонская Ю.Е., Трегубов А.Н., Вергун А.А., Димова А.С., Димов С.К., Янченко Т.А.* Оценка эпизоотического благополучия по бруцеллезу стад крупного рогатого скота при применении живых слабоагглютиногенных вакцин // Ветеринария. 2022. № 1. С. 9–14. DOI: 10.30896/0042-4846.2022.25.01.09-14.
5. *Сакидибиров О.П., Джамбулатов З.М., Баратов М.О.* Кольцевая реакция с молоком для диагностики бруцеллеза у лактирующих коров и коз // Ветеринария. 2020. № 11. С. 10–12 DOI: 10.30896/0042-4846.2020.23.11.10-12.
6. *Халиков А.А, Микаилов М.М., Яникова Э.А., Гулиева А.Т.* Применение РНГА с молоком при диагностике бруцеллеза коров // Ветеринария и кормление. 2020. № 4. С. 50–53. DOI: 10.30917/ATT-VK-1814-9588-2020-4-18.
7. *Димова А.С., Сизов Д.А., Машнин А.В., Воробьев В.И.* Эффективность тест-системы ИФА IDEXX для серологической диагностики бруцеллеза крупного рогатого скота в невакцинированных против данной инфекции стадах // Ветеринария. 2017. № 10. С. 14–16.
8. *Сизов А.А., Димова А.С., Димов С.К., Сизов Д.А., Аракелян П.К., Чекишев В.М.* Эффективность использования О-ПС антигена в ИФА для дифференциальной экспресс-диагностики бруцеллеза крупного рогатого скота // Ветеринария. 2018. № 1. С. 9–14.
9. *Novoa M.B., Aguirre N.P., Valentini B., Torionide-Echaide S., Signorini M.L., Primo M.E., Elena S., Vanzini V.R.* Development, validation and field evaluation of an indirect ELISA for the detection of antibodies against *Brucella abortus* in bulk and individual milk samples in dairy cattle // Preventive Veterinary Medicine. 2022. Vol. 208. P. 105740. DOI: 10.1016/j.prevetmed.2022.105740
10. *Скляров О.Д., Климанов А.И., Калядин Д.В., Шунаева Н.А., Букова Н.К.* Совершенствование теста для дифференциальной диагностики бруцеллеза животных // Ветеринария. 2019. № 1. С. 28–31. DOI: 10.30896/0042-4846.2019.22.1.28-31.
11. *Жанбырбаев М.С., Курбанова А.С., Оспанова М.С., Осербай А.Ж., Курбанова К.С.* Диагностическая эффективность кольцевой реакции при исследовании молока на бруцеллез // Вестник науки Южного Казахстана. 2020. № 1 (9). С. 236–240.
12. *Аракелян П.К., Трегубов А.Н., Руденко А.В., Вергун А.А., Ильин Е.Н., Христенко А.С., Димова А.С., Димов С.К.* Анализ эффективности борьбы с бруцеллезом крупного рогатого скота без вакцинации // Ветеринария. 2019. № 5. С. 9–12. DOI: 10.30896/0042-4846.2019.22.5.9-12.

13. *Аракелян П.К., Трегубов А.Н., Вергун А.А., Руденко А.В., Димова А.С., Димов С.К., Янченко Т.А.* Бруцеллез сельскохозяйственных животных: почему научно управляемая инфекция может быть практически неуправляемой? (научно-аналитический обзор) // Вестник ветеринарии. 2020. № 4 (95). С. 51–58.
14. *Калядин Д.В., Матович Н.А., Чаус В.Ю., Кленов А.С., Вавилова О.В., Моторыгин А.В., Скляров О.Д.* Сравнительное изучение чувствительности серологических тестов при диагностике бруцеллеза животных // Биотика. 2021. № 2 (39). С. 40–44.
15. *Wang Yu., Robertson I., Cheng S., Wang Y., Hou L., Wang G., Wu X., Li X., Chen Y., Guo A.* Evaluation of a milk ELISA as an alternative to a serum ELISA in the determination of the prevalence and incidence of brucellosis in dairy herds in Hubei Province, China // Preventive Veterinary Medicine. 2020. Vol. 182. P. 105086. DOI: 10.1016/j.prevetmed.2020.105086.

REFERENCES

1. Arakelyan P.K., Khristenko N.V., Gaivoronskaya Yu.E., Dimova A.S., Dimov S.K., Yanchenko T.A. Manufacturability of different schemes of immunization of cattle against brucellosis with the possibility of early post-vaccination diagnosis. *Veterinariya = Veterinary medicine,* 2023, no. 5, pp. 11–16. (In Russian). DOI: 10.30896/0042-4846.2023.26.5.11-15.
2. Arakelyan P.K., Gaivoronskaya Yu.E., Rudenko A.V., Ilyin E.N., Dimova A.S., Dimov S.K., Yanchenko T.A. Serological reactivity of healthy sheep immunized against brucellosis with a live vaccine from the B. abortus strain 19. *Veterinariya = Veterinary medicine*, 2022, no. 4, pp. 21–25. (In Russian). DOI: 10.30896/0042-4846.2022.25.4.21-25.
3. Gordienko L.N., Novikov A.N., Kulikova E.V. Effectiveness of the differential test at using in the diagnosis of reindeer brucellosis. *Veterinariya = Veterinary medicine*, 2020, no. 11, pp. 7–10. (In Russian). DOI: 10.30896/0042-4846.2020.23.11.07-10.
4. Arakelyan P.K., Khristenko N.V., Gaivoronskaya Yu.E., Tregubov A.N., Vergun A.A., Dimova A.S., Dimov S.K., Yanchenko T.A. Evaluation of epizootic welfare for brucellosis of cattle herds in conditions of using live weakly agglutinogenic vaccines. *Veterinariya = Veterinary medicine*, 2022, no. 1, pp. 9–14. (In Russian). DOI: 10.30896/0042-4846.2022.25.01.09-14.
5. Sakidibirov O.P., Dzhambulatov Z.M., Baratov M.O. Ring reaction with milk for diagnostics of brucellosis in lactating cows and goats. *Veterinariya = Veterinary medicine*, 2020, no. 11. pp. 10–12. (In Russian). DOI: 10.30896/0042-4846.2020.23.11.10-12.
6. Khalikov A.A., Mikailov M.M., Yanikova E.A., Guliyeva A.T. The use of IHT with milk in the diagnosis of bovine brucellosis. *Veterinariya i kormlenie = Veterinaria i kormlenie*, 2020, no. 4, pp. 50–53. (In Russian). DOI: 10.30917/ATT-VK-1814-9588-2020-4-18.
7. Dimova A.S., Sizov D.A., Mashnin A.V., Vorobyov V.I. Efficiency of the idexx ELISA system for serological diagnostics of brucellosis in non-vaccinated cattle livestock. *Veterinariya = Veterinary medicine*, 2017, no. 10, pp. 14–16. (In Russian).
8. Sizov A.A., Dimova A.S., Dimov S.K., Sizov D.A., Arakelyan P.K., Chekishev V.M. Efficiency of using O-polysaccharide antigen in ELISA for the rapid differential diagnosis of brucellosis in cattle. *Veterinariya = Veterinary medicine*, 2018, no. 1, pp. 9–14. (In Russian).
9. Novoa M.B., Aguirre N.P., Valentini B., Torioni-de-Echay S., Signorini M.L., Primo M.E., Elena S., Vanzini V.R. Development, validation and field evaluation of indirect ELISA for the detection of antibodies against Brucella abortus in mass and individual milk samples from dairy cattle. *Preventive veterinary medicine*, 2022, vol. 208, p. 105740. DOI: 10.1016/j.prevetmed.2022.105740.
10. Sklyarov O.D., Klimanov A.I., Kalyadind D.V., Shunaeva N.A., Bukova N.K. Improvement of the test for differential diagnosis of animals' brucellosis. *Veterinariya = Veterinary medicine*, 2019, no. 1, pp. 28–31. (In Russian). DOI: 10.30896/0042-4846.2019.22.1.28-31.
11. Zhanbyrbaev M.S., Kurbanova A.S., Ospanova M.S., Oserbai A.Zh., Kurbanova K.S. Diagnostic effectiveness of the ring reaction in the study of milk for brucellosis. *Vestnik nauki Yuzhnogo Kazakhstana = Bulletin of Science of Southern Kazakhstan*, 2020, no. 1 (9), pp. 236–240. (In Russian).
12. Arakelyan P.K., Tregubov A.N., Rudenko A.V., Vergun A.A., Ilyin E.N., Khristenko A.S., Dimova A.S., Dimov S.K. Efficiency of combating bovine brucellosis without vaccination. *Veterinariya = Veterinary medicine*, 2019, no. 5, pp. 9–12. (In Russian). DOI: 10.30896/0042-4846.2019.22.5.9-12.
13. Arakelyan P.K., Tregubov A.N., Vergun A.A., Rudenko A.V., Dimova A.S., Dimov S.K., Yanchenko T.A. Brucellosis of farm animals: why a scientifically controlled infection could be

practically non-control? (scientific and analytical review). *Vestnik veterinarii = Vestnik Veterinarii*, 2020, no. 4 (95), pp. 51–58. (In Russian).

14. Kalyadin D.V., Matovich N.A., Chaus V.Yu., Klenov A.S., Vavilova O.V., Motorygin A.V., Sklyarov O.D. Comparative study of the sensitivity of serological tests in the diagnosis of brucellosis of animals. *Biotika = Biotika*, 2021, no. 2 (39), pp. 40–44. (In Russian).
15. Wang Yu., Robertson I., Chen S., Wang Yu., Hou L., Wang G., Wu H., Li H., Chen Yu., Guo A. Evaluation of milk ELISA as an alternative to serum ELISA in determining the prevalence and incidence of brucellosis in dairy herds in Hubei province, China. *Preventive veterinary Medicine*, 2020, vol. 182, p. 105086. DOI: 10.1016/j.prevetmed.2020.105086.

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