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Areas of Concern of Brucellosis Specific Prevention

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Abstract | The epidemiologically justified mass immunization of cattle with live antibrucellosis vaccines in unfavorable areas during the widespread occurrence of brucellosis is currently unreasonable for prosperous regions, from both the environmental and economic point of view. In this regard, the transition to alternative schemes of specific prevention of brucellosis using environmentally safe products is very reasonable. The research was aimed at studying the antiepizootic efficacy of the concomitant use of the antibrucellosis environmentally safe chemical vaccine (ACV) and the vaccine from the Brucella abortus 82 strain at arresting the focus of brucellosis infection. It has been found that the immunization with ACV not only allowed to neutralize the abortogenic properties of the vaccine from the strain 82 but also not to provoke the latent forms of brucellosis reducing thereby the recovery periods of cattle herds from this infectious disease.

Keywords | Brucellosis, Specific prevention, Epizootic situation, Immune-free zone, Strain 82 antibrucellosis vaccine, Abortogenicity, Revaccination, Epizootic efficacy, Environmentally safe chemical vaccine

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INTRODUCTION

In Russia, the historical experience of fighting animal brucellosis for the livestock without the immune background did not lead to the desired results. All efforts aimed at improvement using business management and general veterinary and sanitary measures, as well as regular diagnostic studies, had not provided for the arrest of the infection source since the reinfection of cattle with brucellosis was ahead of the identification of infected animals. In this regard, the epidemiological indicators of human infection with this disease from animals continued to remain at a high level. The economic losses associated both with the violation of the herd reproduction, the cessation of breeding work, and with costly restrictive measures were enormous. Only in the fifties of the last century, the introduction of specific prevention had provided for a sharp decrease in the clinical manifestation of brucellosis, which was reflected in the decrease in the incidence rates of brucellosis in people. However, despite the decrease in epizootological and epidemiological indicators of brucellosis, the final elimination of this infection was not resolved. The main reason was the pronounced agglutinogenic properties of the vaccine widely used at that time from the B. abortus 19 strain, which had made it almost impossible to differentiate the animals reacting to both brucellosis infection and vaccine administration.

In the 1970s and 1980s, a team of scientists from the Siberian Veterinary Research Institute (Starting from 1985, All-Russian Research Institute of Brucellosis and Animal Tuberculosis), the Kazan Veterinary Research Institute, and the Institute of Experimental Veterinary of Siberia and the Far East developed and implemented a scientifically based system of special antibrucellosis measures in cattle, based on the creation of permanent immunity through the use of slightly agglutinogenic antibrucellosis vaccine from the B. avortus 82 strain. The slightly agglutinogenic properties of the vaccine made possible the early postvaccination

diagnostics of brucellosis in the still pronounced immunity stage resulting in rapid recovery of disadvantaged farms. High antiepizootic efficacy of this vaccine manifested itself not only in the Russian Federation but also in a number of USSR countries (Kosilov et al., 1995).

However, it is commonly accepted that the live vaccines used against any infectious diseases involve not only epidemiological and epizootological issues but also environmental ones, including all sorts of complications. Therefore, the world practice is extremely cautious about their use. To a certain extent, such views and attitudes toward vaccine prevention have been confirmed at studying and testing the B. avortus 82 strain vaccine. First of all, it concerns the pronounced abortogenic features of the vaccine, instability of individual biological signs of the vaccine strain as a dissociant, the potential for its reversion to the original virulent S-form by the R → RS → SR → S type.

The negative effects of the brucellosis vaccines using live vaccines (especially multiple ones) have also been noted in epidemiology. In this case, it is appropriate to make epidemiological observations indicating the negative effects of vaccination of people against brucellosis (especially multiple ones), expressed in postvaccination pathergy (Kosilov et al., 1995). The authors note that immunization of people causes a number of adverse reactions and even diseases among those vaccinated. Annual revaccination of the population led to the formation of an immunoallergic background with hypersensitization phenomena, symptoms of "vaccination pathergy" appeared on the organismic level, which did not differ from those in latent and chronic forms of brucellosis (Osterman and Moriyon, 2006).

Kosilov et al. (1999) have found that repeated revaccination of animals can cause severe pathology at the tissue-organ level from different organs and systems, affecting their productivity. In addition, so far, the effects of the long-term persistence of brucellous antigen, including the vaccine one, which is accompanied by adaptation, reproduction, and death of microbial cells, destruction and circulation of the released soluble antigens, have not been sufficiently studied. Only highly sensitive diagnostic methods (ELISA, PCR) have allowed to show that the persistence of brucellous antigen is much longer than it has previously been admitted (Zheludkov et al., 2003; Erganis et al., 2005).

The immunoprophylaxis regulation recommended by the instructions, covering all sex and age groups of livestock, including regular reimmunization of cows at intervals of one to two years, was adequate to the epizootic situation that had been developed in the 1970s and 1980s (Kosilov et al., 1999). At the same time, the visible advances in the treatment of brucellosis in animals, which are focused on clinical and serological indicators, overshadowed the environmental aspects of mass immunization with live anti-brucellosis vaccine and its negative consequences.

To date, in more than 20 years, significant changes have occurred towards the fundamental improvement in the epizootic situation, which no longer requires such strict regulations for specific prevention, taking environmental problems into account. At first glance, it would seem that the cessation or reduction of the antigenic load on the organism of animals is the simplest and most natural solution to the environmental problem associated with the vaccine prevention of brucellosis. However, the occurrence of brucellosis in certain territories, even sanitazed ones, and especially cases of human morbidity, require extreme caution when dealing with this issue. In this regard, it is necessary to find alternative specific prevention schemes with the use of more environmentally safe biological products, which differ favorably from the strain 82 vaccine (Novitskiy et al., 2016).

Considering the environmental issues related to the use of live vaccines, a number of researchers began to research and test new generation vaccine formulations made not only from inactivated strains of microorganisms (nonliving vaccines) but also from individual components extracted from them (chemical vaccines). Own results of laboratory and production tests of inactivated ACV, developed at the Siberian Veterinary Research Institute and representing the insoluble Brucella antigen complex, convinced the authors of the expediency of such line of research (Novitskiy and Okolelov, 2016; Popova et al., 2006). The use of ACV allowed to not only prevent brucellosis, but also to sanitate some farms in the Omsk Region. The use of such vaccines is most justified in relatively safe areas to maintain the immune status of animals during the transition to guaranteed wellness, followed by the cessation of specific prevention.

To date, due to the scientifically based system of special antibrucellosis measures, brucellosis of farm animals has moved into the category of controlled infections making it possible to completely sanitate the regions of Western Siberia. Nevertheless, the issues of specific prevention of brucellosis continue to be relevant, since the threat of its penetration persists, primarily from the CIS adjacent territories.

MATERIAL AND METHODS

The research was carried out in one of the farms of the North Kazakhstan region under the international cooperation agreement between the Omsk State Agrarian University and the North Kazakhstan Research Institute of Agriculture.

The studies were carried out on 1,200 beef-cattle animals, placed on four branches of the farm. During the last three years, the animals have not been subjected to antibrucellosis vaccination. In order to prevent brucellosis, identify latently sick animals and prevent postvaccination induced labors from the subsequent use of the strain 82 vaccine on the entire population, the ACV was used after the preliminary study.

Based on the fact that immunostimulation with a chemical vaccine provokes an immune response in hidden brucellosis carriers in a short period after its use, postvaccination serological studies of blood serum were performed in a short time after 21 days, 2, 3 and 5 months using agglutination reaction (AR), rose bengal test (RBT), and immunodiffusion reactions with O-polysaccharide antigen (IDR with O-PS-antigen) as per the brucellosis diagnosis instruction (Manual on the diagnosis of animal brucellosis, 2003). Upon recovery in order to increase the immunity stress, the entire livestock of the farm was reimmunized with the strain 82 vaccine.

RESULTS AND DISCUSSION

According to official data, the farm in which the research work was started had been considered safe by the cattle brucellosis at that time. The cows had been previously immunized with the strain 82 vaccine more than three years ago. There were no animals reacting to brucellosis before vaccination. However, after another study of blood serum in the district veterinary laboratory using enzyme-linked immunosorbent assay (ELISA), 30 cows responding to brucellosis were isolated at Section 1. In order to clarify the epizootic situation of brucellosis in all sections, studies were carried out followed by the use of ACV on all sex and age groups. The use of ACV in specific prevention allowed provoking immune reactions in brucellosis carriers with a latent form of brucellosis, to improve the immune status of animals, and prevent the appearance of postvaccinal induced labors from subsequent vaccinations with a strain 82 vaccine. Other researchers also reported on the provocation of latent forms of brucellosis using antibrucellosis vaccine (Ivanov, 2007; Novitskiy and Okolelov, 2016).

The results of postvaccination studies of animals placed on four sections of the farm are presented in five tables. As can be seen from the tables below, the epizootic situation in different sections varied considerably.

Table 1 shows the results of the study of 30 cows reacting by ELISA, as well as the nursing calves, kept with them.

The data in the table show that as a result of the study of the blood serum, before the ACV introduction, no one responded to any of the serological reactions used, although the cows reacted by ELISA. These data also indicate that the use of the highly sensitive ELISA method in animals even in the long-term after the immunization with the strain 82 vaccine (3 years) is problematic since the individuals that carry postvaccination antibodies were detected. After using ACV, antibodies were detected only in low titers in individual animals and in a short time (21 days): RA–50, 100 ME, and CFT–only 1:5, indicating their postvaccination nature. In this case the IDR with O-PS-antigen was used as the differential test, which had shown a negative result, indicating the wellness of Section 1 for brucellosis. These data confirm the reports of a number of researchers about the significance of IDR indicators in the epizootic assessment of herds for brucellosis.

Table 1: The results of the serological study of the ELISA-positive cows and nursing calves of cattle for brucellosis before and after ACV vaccination (Section 1).

Group	Number of animals	Term of the study	Results of serological reactions					
			IDR	RA, ME			CFT titers	
				50	100	200	5	10
Before the ACV introduction								
Cows	30	Not vacc.	-	-	-	-	-	-
Calves	50	Not vacc.	-	-	-	-	-	-
After the ACV introduction								
Cows	30	21 days	-	10	2	-	15	-
Calves	53	21 days	-	5	1	-	17	-
Cows	30	2 months	-	-	-	-	-	-
Calves	58	2 months		-	-	-	-	-

A somewhat different picture was observed in Section 2, where in the study of serum among cows, positively reacting animals with high RA and CFT titers and positive IDR had been detected. ACV was applied after the removal of the reacting animals. The results of the study before and after the vaccine introduction are shown in Table 2.

Table 2: Serological studies of blood serum before and after the ACV introduction (Section 2).

Number of animals	Term of the study	Results of serological reactions						
		IDR	RA, ME			CFT titers		
			50	100	200	5	10	20
206	Not vacc.	6	18	4	4	12	6	5
First introduction of drug								
195	21 days	-	27	79	10	21	29	32
174	2 months	1	24	18	3	27	16	1
168	3 months	0	5	2	0	0	0	0
Second drug introduction after three months								
154	21 days	4	31	28	3	23	19	7
158	2 months	0	10	8	2	5	2	0

The above table shows the provoking properties of ACV, which on day 21 after the use, allowed to further isolate animals with a positive reaction to brucellosis, which had been removed. Further studies of livestock after two and three months showed a marked decrease in the number of reacting animals. Three months later, animals with indicators of Brucella infection were no longer registered, after which the ACV was reintroduced. Reintroduction again triggered a spike in reactions in the remaining carriers of Brucella infection. On day 21, four cows with positive IDR, four with high RA titers, and nine with positive CFT in high dilution were allocated and removed from the herd.

At Section 3, the epizootic brucellosis situation was manifested by clinical course (induced labors). To arrest the spread of infection, ACV was applied twice with an interval of three months.

The results of the study before and after the ACV introduction are shown in Table 3.

Table 3: Serological studies of blood serum of the cows after the ACV vaccinations in the herd with clinical manifestation of brucellosis (Section 3).

Number of animals	Term of the study	Results of serological reactions						
		IDR	RA, ME			CFT titers		
			50	100	200	5	10	20
204	8 months	14	34	5	7	16	10	6
ACV revaccination								
185	21 days	14	14	49	39	5	41	39
169	2 months	4	22	12	5/4	38	21	6
155	3 months	0	29	4	0	8	2	0
Revaccination with ACV after three months								
154	21 days	2	34	17	3	23	18	13
152	2 months	0	10	8	0	5	2	0

The data in the table show that the largest number of latently sick animals was detected only after the ACV provocation. Two-time study after the revaccination after 21 days and two months with the removal of reactants allowed to practically achieve the sanitation of the herd in a short time, which was confirmed by the absence of reacting IRD and RA, CFT in high titers. In another study, two months later, only animals with postvaccination reactions in RA and CFT were recorded.

Analysis of the results of studying the cows in Section 4 has confirmed the importance of using the ACV provoking properties. This way, while seven months after the initial use of ACV, only 1 % of reactive IRDs had been recorded, after 14 days and two months after the RIDs provocation, the number of positive reactions increased to 8 % (Table 4).

The provoking properties of the chemical vaccine were also studied in the research of heifers and bred heifers before insemination (Table 5).

It can be seen from the table that out of 218 IRD-studied animals, only three animals (2.8 %) with brucellosis were revealed, whereas three months after the ACV provocation–12 (11.4 %).

After achieving the indicators characterizing the absence of Brucella infection (negative IRD with O-PS antigen, low RA and CFT titers), all the livestock of cows in the farm, excluding the period of pregnancy, were immunized with B. Abortus 82 strain vaccine. In the observation of the vaccinated animals in the postvaccination period, there were no cases of induced labors, which also indicated the ACV properties neutralizing the abortogenicity of the strain 82 vaccine.

DISCUSSION

The results of personal studies have confirmed that the assessment of the epizootic situation for brucellosis in herds, where specific prevention was carried out, should be based on RA and CFT indicators and IRD with O-PS antigen. The use of ELISA for this purpose is not justified, since due to its high sensitivity this method detects not only post-infectious but also postvaccination antibodies.

When solving the problems of herd sanitation, it is necessary to take into account the features of the pathogenesis of brucellosis, namely the lack of an immune response in certain phases of its development (adaptation phases, regional infection and secondary latency). As a means capable of causing a secondary immune response in hidden carriers of the infection, ACV was used, having both immunogenic and provocative properties.

Studies of disadvantaged herds in a short time after the use of ACV allowed identifying animals reacting in the IRD, as well as in the RA, CFT in diagnostic titers, which confirmed the provoking properties of a chemical vaccine. Responsive animals have been removed. The subsequent studies after the reintroduction of this vaccine showed a sharp reduction in sick cattle.

Thereafter, IRD with OP-C antigen was used as a criterion for assessing the herd sanitation. The lack of positive indicators of this reaction in the herd as a whole testified to its wellness.

The negative properties of the vaccine from strain 82, associated with its abortogenicity, are neutralized by pre-immunization of cows with ACV.

Table 4: Serological studies of cows (Section 4) after the ACV provocation.

Group	Number of animals	Term of the study	Results of serological reactions						
			IDR	RA, ME			CFT titers		
				50	100	200	5	10	20 and higher
Cows	291	7 months	3	24	14	2	10	2	1
ACV									
Cows	86	7 days	0	7	5	1	15	3	-
Cows	267	14 days	12	58	103	46	67	32	23
Cows	332	21 days	9	81	74	94	49	23	12
c+heifers	499	1.5 – 2 m	4	87	49	0	29	9	3
Cows	344	4 – 5 months	2	63	24	6	50	14	5

Table 5: Serological studies of heifers and bred heifers of a similar age after the ACV provocation.

Group	Number of animals	Term of the study	Results of serological reactions						
			IDR	RA, ME			CFT titers		
				50	100	200	5	10	20
bred heifers	83	8 months	1	-	-	-	1	-	-
heifers bef/insem	125	8 months	2	3	1	0	3	2	-
ACV immunostimulation									
heifers bef/insem	105	3 months	12	13	12	5	4	7	6/3/1
heifers bef/insem.	100	4 months	2	15	1	2/1	15	4	2/1

Note: heifers bef/insem – heifers before insemination.

CONCLUSION

The article materials indicate the need for the differentiated approach in the system of special antibrucellosis measures. First of all, it is necessary to take into account the epizootic situation at the regional level, the existing threat of infection, the timing of recovery and stability of wellness in terms of brucellosis. In the ecological and economic terms, the specific prevention of brucellosis should be considered as a forced measure that should be used only in case of emergence. Upon reaching wellness on the individual zones or regions scale, the antigenic load of live antibrucellosis vaccines should be reduced and, for the transitional period, to the full rejection of specific prevention, environmentally friendly vaccine formulations should be used.

When evaluating the antiepizootic efficacy of the concomitant use of the B. abortus 82 strain and ACV, it has been established that the immune background created with the help of the chemical vaccine allows neutralizing the abortogenic properties of the vaccine from the 82 strain. Moreover, ACV can be used as a means of provoking latent forms of brucellosis.

During the rehabilitation of farms with the use of serological methods of diagnosis, the presence of brucellosis carriers with latent forms of infection in the herds should be taken into account, which requires multiple repeated studies. The farm recovery terms can be significantly reduced by using the provoking properties of environmentally friendly antibrucellosis vaccines for this purpose.

AUTHORS CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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