

Ways of Antiepidemiological Measures' Improvement in Case of Cattle Brucellosis



V.I. Piontkovsky, A.M. Ergazina, V.A. Sapa, G.K. Murzakaeva, E.O. Rudenko

Abstract: The paper discusses epizootic situation of cattle brucellosis in the Republic of Kazakhstan and the subjects of the Kostanai region. The system of comprehensive anti-brucellosis measures based on the major principles with integrated organizational, economic, general veterinary and sanitary and special measures using specific prevention means according to certain schemes or without them, depending on the current epizootic situation, which transfers brucellosis into the category of controlled or potentially curable ones, is analyzed. Convincing evidence of the role of these factors in relation to the success of prevention and rehabilitation of infected herds is provided.

The long-term research results indicate that in the regions largely affected by brucellosis complete recovery of cattle without specific prevention measures is practically impossible.

In this regard, to eliminate the risk of infection we proposed schemes of anti-brucellosis immunization in safe regions and the subjects affected to different degrees, as well as that for imported pedigree cattle and its offspring. The schemes stipulate vaccines (dosage, frequency, place of immunization, intervals, timing of revaccination, etc.), timing and methods of examination different gender and age groups of cattle.

Keywords: brucellosis, vaccines, diagnosis, epizootic situation, prevention. immunization scheme, immunity, revaccination.

I. INTRODUCTION

Brucellosis of animals, including cattle, remains one of the most difficult problems of infectious pathology. The characteristic features of the infectious process are: occurrence in many countries, ability of pathogens to live in the body of various types of living creatures, latent course, complexity of diagnosis and specific prevention measures, as well as a long incubation period [1-3]. Each subsequent sick animal becomes a source (producer) of the pathogen, releases it into the environment through all the secrets and excreta, and infects healthy animals. Economic damage is manifested in reduced productivity, abortion, premature culling of highly

productive animals, as well as in the enormous cost of a complex of organizational, economic, veterinary, sanitary and special measures [4]. This disease, being extremely dangerous for people, causes particular concern and often leads to temporary or life-long disability [5, 6].

Currently, the genus *Brucella*, taking into account the infection of different species of animals and humans, includes ten species: *Br. melitensis*, *Br. abortus*, *Br. suis*, *Br. canis*, *Br. ovis*, *Br. neotomae*, *Br. ceti* (cetacean brucellosis pathogen), *Br. pinnipedialis* (pinnipeds brucellosis pathogen), *Br. microti* (gray vole brucellosis pathogen) and *Br. inopinata* (brucellosis pathogen isolated from the breast implant of a sick woman). The first three types of brucella according to a number of distinguishing features are divided into biotypes: *Br. melitensis-3*, *Br. abortus-8*, *Br. suis-5* [5]. Many of them are pathogenic for humans [2, 7, 8].

II. PROPOSED METHODOLOGY

The Kostanai region is hazardous for brucellosis of animals and, first of all, of cattle brucellosis. Brucellosis, as a new and little-studied disease in cattle, was first registered in the year of formation of the region in 1936. Then 24 hazardous sites were discovered and 673 reactive animals out of 16.3 thousand examined were serologically identified. The percentage of infection was 4.12. In the next two years the number of hazardous sites increased to 66, in which 2.2 thousand out of 118.0 thousand serologically examined animals were registered. The incidence rate was 1.7 (1937) and 2.0 (1938) [9]. In subsequent years, according to official veterinary statistics on cattle brucellosis epizootic situation remained complex and sometimes tense both in the Republic of Kazakhstan and in the subjects of the Kostanai region. The main reasons for the spread of this disease among animals were disregard of strict veterinary and sanitary rules; a shortage of veterinary specialists and insufficient coverage of animals with diagnostic tests, including stud bulls; untimely isolation and delivery of sick cattle for slaughter; violations of preventive and ongoing forced disinfection and disregard of checking their quality; non-standardized conditions of maintenance and proper feeding, feeding the calves with non-neutralized milk and skim milk as well as the formation of the herds with the inclusion of young animals born to positively reacting to brucellosis cows and heifers, among which undoubtedly there were tolerant animals. The latter animals are not detected by generally accepted standard diagnostic methods; they remain in the herds as brucella carriers, that is, they are hidden sources of infection and thus they support epizootic process [2, 10, 11].

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The official veterinary reporting data on cattle brucellosis both in the Republic of Kazakhstan as a whole and in the subjects of the Kostanai region over the past twenty years (1997-2017) reveal an alarming epizootic situation and do not give ground for a favorable prognosis.

Thus, the number of responders (affected animals) to cattle brucellosis in Kazakhstan ranged from 2.44 (2013) to 129.8 (2009) thousand heads, and the percentage of infection was from 0.4 to 1.8%. In 2017, the number of animals reacting to brucellosis was 39.2 thousand animals, and the percentage of infection was 0.78. According to the subjects of the Kostanai region, the number of reacting animals in 2017 compared to the similar indicators in 1997 decreased 4.2 times, and the percentage of infection more than 8.7 times. Prevention and rehabilitation of cattle from brucellosis in Kazakhstan for seven years (2007-2013) was carried out without the use of specific preventive measures. The ban on vaccination of cattle and the treatment of brucellosis transferred the prevention of this disease to the plane of administrative, organizational, economic and sanitary measures. It was during this period that the number of animals responding to brucellosis increased 53 times in the republic, from 2.44 to 129.8 thousand animals, and the percentage of infection 4.5 times, from 0.4 to 1.8. The number of reacting animals in 2007 in the Kostanai region in comparison with 1997 decreased 4.2 times, and the percentage of infection - more than 8.7 times. In 2008 (one year after the ban on vaccination) the percentage of infection increased 6 times and amounted to 1.08, and in 2009 the figures are respectively 10.8 and 1.94. The number of animals reacting to brucellosis that year increased to 12.2 thousand animals, which is 9.17 and 2.36 times higher than the level of it in 2007-2008 respectively. A relatively high rate of cattle responding to brucellosis was also recorded in 2010 (7.75 thousand animals). In the subsequent 2011-2013 the number of animals reacting to brucellosis decreased 1.4-1.7 times, and the percentage of infection decreased 2.2-3.0 times and amounted to 0.72% in 2013. In 2016, the number of cattle reacting to brucellosis remained at a rather high level and amounted to 7.68 thousand, and the percentage of infection was 1.42. Of the animals that react to brucellosis 73.88% are breeding stock, 24.16% are the remaining adult stock and 1.96% are stud bulls. The number of recent cases of infected people, according to the RTD "Department of Public Health of the Kostanai Region of the Ministry of Health of the Republic of Kazakhstan", amounted to 35 in 2016 and 22 in 2017. Reactions to brucellosis by serology are also recorded in other types of farm and domestic animals: in horses from 0.08 to 0.36%, in camels from 1.44 to 2.6%, in pigs from 0 to 0.02% and in carnivores from 0.2 to 0.49%. All animals reacting to brucellosis were disposed of and the products from farm animals were processed at a security enterprise.

III. RESULT ANALYSIS

We have already mentioned that the prevention and rehabilitation of cattle for seven years (2007-2013) was carried out without the use of specific preventive measures. In connection with this we developed and tested a system of preventive measures for brucellosis in cattle without the use of anti-brucellosis vaccines, the essence of which was in

universally protecting the subjects from the introduction of brucellosis pathogens; continuous organizational, economic, general veterinary and sanitary and special diagnostic measures, at least two serological tests for brucellosis; two preventive disinfections (in the spring after pasturing cattle for summer pasture maintenance and in autumn - before setting the cattle for stall maintenance). At the same time animals were injected with ivomec or other effective antiparasitic drugs (cidectin, ivermectin, etc. according to the instructions) to reduce or eliminate the associative manifestation of parasites that affect the functioning of animal's immune system and lead to the development of pathological conditions of various degrees, including immunodeficiency. This approach should at the first stage remove the allergenic, toxic and immunosuppressive effects of helminths (diticulosis, strongylatoses, trichocephaliasis and other nematodes) and parasites (psoroptosis, syphunculosis, hypodermatosis, teliiasis, etc.), at the second stage in a month after deworming to conduct serological (brucellosis, chlamydia, leukemia, listeriosis, leptospirosis) and allergic diagnostic tests (tuberculosis), and at the final stage - the necessary routine vaccinations according to the current epizootic situation. The intervals between scheduled vaccinations should be at least 12-14 days. The proposed system does not contradict the general rules, requirements of provisions and instructions for all nosological diseases of the Veterinary Legislation of the Republic of Kazakhstan, but provides for the observance of the sequence of planned antiepzootic treatments in order to create optimal conditions for maximal application of immunological capabilities of animal bodies. The introduction of this system into the veterinary practice of agricultural units of the Taranovsky district is economically justified and allowed reducing the number of animals reacting to brucellosis 1.4-3.9 times [2]. Currently, agricultural organizations in this region are preventing bovine brucellosis without the use of anti-brucellosis vaccines. Many years of experience in combating brucellosis infection revealed that in farms and regions heavily affected by the disease the rehabilitation of cattle and other animal species without the use of specific preventive measures is very difficult and sometimes just impossible [12, 13]. That is why, depending on the current epizootic situation in cattle brucellosis, we propose optimal immunization schemes for the formation of a promising comprehensive system for the prevention and control of brucellosis using registered in the Republic of Kazakhstan and the countries of the Customs Union anti-brucellosis vaccines.

1. In cattle brucellosis-free farms one should apply the 82 + 82 + 82 scheme:

- heifers of 3-6 months of age should be examined by enzyme-linked immunoassay (ELISA) or rosenalprobe (RBP), agglutination test (RA) and complement fixation reaction (CSC). Those not reacting to brucellosis should be inoculated with a full dose of live vaccine from a weakly agglutinogenic Br abortus 82 strain. In subjects that have been stable for brucellosis for 3 years or more young animals are vaccinated without preliminary serological testing for brucellosis;

- mature heifers 2-3 months before insemination, but not earlier than 10 months after primary vaccination, are serologically examined by classical methods and those not responding to brucellosis are revaccinated with the full dose of the same vaccine from strain 82;

- cows, 1-2 months after calving of the whole herd, should be examined with classical methods for brucellosis (RBP, RA, CSC) and those not responding annually for 3-4 years are revaccinated with a full dose of vaccine from strain 82 to achieve sustainable well-being;

- stud bulls are to be examined using classic methods on a quarterly basis; they should not be vaccinated. Bulls for rearing and pedigree should not be vaccinated as well.

2. In cattle brucellosis hazardous agricultural units with a percentage of incidence of brucellosis of up to two or more vaccination should be carried out according to the scheme described above 82 + 82 + 82 or 19 + 82 + 82:

Young animals of 3-6 months should be examined with ELISA or classical methods (RBP, RA, CSC) and non-responsive animals should be immunized with a full dose of live anti-brucellosis vaccine from strain Br. abortus 19 in accordance with the current instructions for its use with verification for the titer in 21 days. This will allow identifying brucellosis-tolerant heifers born to brucellosis-infected cows and those heifers, who were infected in utero or postnatally. It will make it possible to prevent these sources (heifers) from further reproduction. Quite often, if not every time, cows and heifers reacting to brucellosis are handed over to forced slaughter and heifers born to these cows are used for further reproduction.

Adult heifers 2-3 months before insemination, but not earlier than after 10 months after primary vaccination, should be serologically examined for brucellosis and non-responsive ones are to be revaccinated with live weakly glutenic vaccine from Br. abortus 82 strain in full dose.

15-21 days after calving cows should be preliminarily examined with an allergic test by the KazNIVI allergen, according to the instructions, and then serologically (RBP, RA, CSC) examined as well after the same period (15-21 days). An allergic test for intradermal application does not have sensitizing properties, but provokes latent forms of the course of brucellosis, which are subsequently captured by serological studies [13]. Non-responsive animals are revaccinated annually for 3-4 years with a slightly agglutinogenic vaccine from strain 82 in a full dose to achieve sustainable well-being. Animals after revaccination are examined within the time period provided for by the instructions for its use. Revaccination of adult heifers before insemination and cows with the vaccine from strain 82 against the background of vaccination of young animals with the anti-brucellosis vaccine from strain 19 will contribute to the formation of more intense immunity.

The use of live anti-brucellosis vaccine Br. abortus from strain 19 in young animals of 3-6 months poses no danger. Post-vaccination antibody titers fade in them within 10 months, in contrast to its use in adult animals, in which case post-vaccination antibody titers accumulate in high titers and last much longer. In case of revaccination the situation persists for years, which causes certain difficulties in their differentiation and an assessment of an epizootic situation. For this reason adult cattle should not be vaccinated with the

vaccine from strain 19.

Stud bulls should be tested with serological methods on a quarterly basis, but they should not be exposed to vaccination.

3. Cattle purchased under the state program for breeding purposes from non-CIS countries (USA, Canada, Austria, France, Australia, Germany, Belgium, Hungary, etc.) and imported into the Kostanai region are usually vaccinated at a young age (4-10 months.) with American vaccine Br. abortus from strain RB-51, which replaced the vaccine from strain 19.Br. abortus from strain RB-51 in the US market. It is a genetically stable mutant which lacks in lipopolysaccharide (LPS) of the O-side chains on the surface of the brucella responsible for the production of S-diagnostic antibodies against brucellosis infection in animals, which means that this vaccine does not stimulate the synthesis of antibodies for standard S-diagnostic tests (RBP, RA, RSK, RDSK, ELISA). Animals vaccinated with this vaccine can be examined at any time after vaccination. The vaccine is registered in the Register of Veterinary Medicines and is approved for use by VCC and N Ministry of Agriculture of the Republic of Kazakhstan since November 2012 according to the approved instruction (01.12.2012).

Due to difficult epizootic situation regarding brucellosis and the elimination of the risk of infection of imported livestock in a complex of measures a dry live vaccine for cattle brucellosis Br. abortus strain RB-51 was used in the subjects of the Kostanai region. It was delivered in the following way as per instruction: young animals aged 4-6 months were examined for brucellosis with an enzyme immunoassay or with classical methods and non-responsive animals were to be immunized; heifers aged 10-16 months were serologically examined and those reacting negatively were revaccinated. Vaccination and revaccination of adult animals at hazardous zones is allowed. The vaccine dose of 2 ml for all sex and age groups of livestock is administered subcutaneously in the posterior third of the neck. Initially guided by the instruction and commercials: "The vaccine RB-51 is the best protection against brucellosis!"; "The RB-51 vaccine is a reliable protection against brucellosis!", this vaccine manufactured by Colorado Serum Company (USA) was used for preventive purposes and out of necessity in the overwhelming majority of agricultural units of the region for imported and local cattle. In a number of subjects the use of the indicated vaccine did not produce the desired effect and further rehabilitation of cattle was carried out according to the scheme 82 + 82 + 82. The mentioned above units include "Saryagash LLP" and "Krymsky" of the Denisov district, "Zlatoust", "Krylovsky" of the Sarykol district, "Dokuchaevsky", "Silant'yevka" of the Altynsarin district, "Aman-Tersek", "Tobolsk", "Moskalevsky" of the Auliekol district, "Shili" of the Naurzumsky district, "Boscol-Astyk" of the Karabalyk district, "Arzamas" of the Uzunkolsky district and many others which are currently considered non-hazardous.

The RB-51 vaccine is new both for veterinary specialists of Kazakhstan and for the subjects of the Kostanai region and many aspects of its application in the general complex of anti-brucellosis measures are not clear yet:

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- the supplied series of this vaccine are not bacteriologically controlled but such state control should be carried out at least at the level of the republican veterinary laboratory or reference laboratory;

- lack of affordable control over its use. Vaccinated animals do not respond to our diagnostic tests (RBP, RA, RSK, RDSK, ELISA) and the R-antigen is not included in the kit;

- there is no reliable control of the temperature regime of storage during the delivery period. Its activity is not guaranteed at + 8 ° C and the use is banned;

- it is advisable to use the vaccine from 4-6 months of age and revaccinate the animals at the age of 10-16 months. According to the commercial vaccination is to be given from 3 months once and revaccination of adult animals is allowed if there is a risk of infection. The latter always exists in a hazardous zone. And what is the time frame of cows' revaccination? Should they be revaccinated in a year, in two years or annually? ;

- after application of the vaccine RB-51 in the general complex of antiepidemiological measures for animals negatively responding to serology to brucellosis, animals reacting to brucellosis are detected at different subsequent periods of the study, which indicates the possibility of provoking latent forms of brucellosis that are captured in the future by legalized serological tests. In all likelihood this phenomenon became the reason for the further prevention and rehabilitation of cattle from brucellosis using the Br. abortus vaccine from strain 82 in a complex of measures.

From the given above data it follows that the manual on RB-51 requires significant amendments and clarifications as well as its interconnections with the existing directive documents on veterinary medicine. We hope that conducting commission tests as well as an objective analysis of preventive and rehabilitation of anti-brucellosis measures using the RB-51 vaccine in different regions of the republic will fill in the gaps.

4. Imported cattle from the Russian Federation should be examined and immunized with anti-brucellosis mildly agglutinogenic vaccine from strain Br. abortus 82 according to the scheme (82 + 82 + 82) or other schemes depending on the epidemiological situation in brucellosis of cattle of those subjects where they were placed.

5. In subjects of different forms of ownership in which they practice year-round calving and in herds there is always a livestock in different stages of pregnancy we propose to take fractional doses of the vaccine from strain Br. abortus 82 to prevent abortion and create immunity. Heifers of 3-6 months were tested with ELISA before vaccination, and adult heifers and cows - with classical methods (RBP, RA, CSC).

Heifers of 3-6 months, adult heifers 2-3 months before insemination and non-pregnant cows were vaccinated in full doses (according to the instructions). Diagnostic serological studies in primary vaccinated heifers should be carried out with classical methods in 10 months after immunization and the animals not reacting to brucellosis should be revaccinated with the same vaccine in a full dose. Cows are finally tested serologically for brucellosis 1-2 months after calving of the brood stock of the whole herd.

The effectiveness of this scheme was tested on the basis of

"Buildservice" LLP in the Kostanai district. In this agricultural unit cattle of the Kazakh white-headed ("Friendship" unit) and black-mottled breed ("Zhdanovka" unit) are being bred. By the beginning of the work (September 2016) on the use of a live dry vaccine against brucellosis from a weakly agglutinogenic strain of Br. abortus 82 in the general complex of anti-brucellosis measures 18 cows reacting to brucellosis were isolated and put to slaughter. It was established that among the breeding stock, more than 74.0% were at different stages of pregnancy, including 12% up to 3-4 months, 28.5% - up to 5 months, 20.25% - up to 6, 12% - up to 7, 2% - more than 8 months. Given the current situation as well as the lack of immune background in animals we used a vaccine from strain Br. abortus 82 according to the following procedure. A vaccine in one hundredth dose containing 1 billion microbial cells was subcutaneously injected to the cows and heifers at different stages of pregnancy and in two months instead of the full dose they were given a dose of 5 billion microbial bodies. Abortions of pregnant animals were not registered while using the scheme. In addition to this permanent immunity is created in animals, it does not interfere with the annual diagnostic tests and, in combination with other measures, provides quick rehabilitation of cattle herds from brucellosis infection. The agricultural unit was rehabilitated from cattle brucellosis in 2017. The same vaccine administration scheme was used for the prevention of brucellosis in already healthy cattle in 2017-2018. The work in this direction is still in progress.

6. In some cases when for some reasons it is not possible to use live brucellosis vaccines due to abortogenicity, in order to create primary (basic) immunity in cattle, in coordination with the regional territorial inspection of VCC and N Ministry of Agriculture of the Republic of Kazakhstan, non-living brucellosis KazNIVI vaccine can be used for rehabilitation. It is completely safe and creates a fairly intense immunity. Post-vaccination antibodies disappear from the blood serum in 3-4 months and diagnostic studies are carried out 6 months after the vaccination. The vaccine can be used both individually and in combination with antibacterial drugs and other anti-brucellosis vaccines [14].

Vaccinated with any vaccine from the above mentioned schemes animals are marked at the base of the right ear with special tags or plucks. In the passports of the animals the records are made.

To differentiate manifested serological post-vaccine reactions from spontaneous ones, especially if the study terms are not observed, one should use the immunodiffusion reaction (RID) with O-PS antigen, and in dairy cows - a ring reaction with milk (CRM). The decision about taking specific preventive measures against brucellosis in animals, including cattle, is made by the authority of the district veterinary service in coordination with the regional territorial inspection of VCC, N Ministry of Agriculture of the Republic of Kazakhstan and the authorized body of VCC and N Ministry of Agriculture of the Republic of Kazakhstan. It is also advisable to obtain scientific support of the scientists or experienced specialists in infectious pathology, agreed upon with the authority of the territorial inspection.

The choice of anti-brucellosis vaccine and its application for specific prevention should be differentiated with regards to epizootic situation of the economic entity.

IV. CONCLUSION

Thus, a system of comprehensive anti-brucellosis measures based on major principles together with organizational, economic, general veterinary-sanitary and special measures using registered means of specific prevention according to certain schemes or without them (specific brucellosis vaccines) depending on the prevailing epizootic situation transfers brucellosis into the category of controlled and therefore into the category of eradicated diseases. The same system should ensure epizootic well-being and guarantee successful implementation of a number of targeted programmes for the development of livestock production in Kazakhstan, aimed at providing environmentally friendly, safe and high-quality livestock products for both domestic and foreign markets.

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