

ANNOTATION

of the dissertation work of Aitkulova Ayaula Mukhametkaliyeva on the topic "Immunological efficacy of conjunctival use of the vaccine from pieces *B. abortus 19* against brucellosis cattle" submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D120100 - "Veterinary medicine".

Relevance of the research topic. Brucellosis - is a chronic infectious disease characterized by abortions, retention of placenta, endometritis, orchitis and sexual dysfunction of animals. Premature slaughter of infected animals for meat before the end of the period of economic use, abortions of dams, and measures to improve farms that are not safe for brucellosis bring large economic costs to farms. In addition, brucellosis is a serious social problem, as people also suffer from it. The main and effective way to prevent brucellosis is pre-immunization of animals. Studies of the effectiveness of vaccines used against brucellosis have shown that they are different. According to scientists, the most optimal for practical use at the present time is the introduction of the *B. abortus 19* vaccine into the conjunctiva. This approach, in addition to creating immunity to brucellosis, makes it possible to conduct scheduled diagnostic studies in a timely manner.

In order to use this method in practice, one of the most urgent tasks of modern veterinary medicine is the study and recommendations on the use of optimal immunization methods.

The purpose of the dissertation research:

- the aim is to analyze the epidemic situation of brucellosis of animals in the territory of the Republic of Kazakhstan in recent years, to study the immunological effectiveness of the *B. abortus 19* vaccine for conjunctival injection and to recommend it for use at farms.

Research objectives:

1. Analyze the epizootological situation of animal brucellosis in the territory of the Republic of Kazakhstan in recent years.
2. Determine the effectiveness of the method of multilocus analysis of variable number of tandem repeats (MLVA-16) for the identification of *Brucella* strains in order to control the sources of infection.
3. Evaluate the effectiveness of specific measures to combat brucellosis in animals in the Republic of Kazakhstan over the past years.
4. Determine the immune status of guinea pigs vaccinated with different doses and methods of *B. abortus 19* vaccine against brucellosis
5. Study of the post-vaccination dynamics of antibodies in cattle vaccinated with various amounts and methods of *B. abortus 19* vaccine.
6. Control of the epizootic efficiency of the *B. abortus 19* vaccine into the conjunctiva injection method in a production environment.
7. Develop a proposal for the use of *B. abortus 19* vaccine against brucellosis in cattle by the conjunctival method.

Materials and methods of research. As research materials, pathological material obtained from animals (blood, milk, blood serum, abortions, sections of parenchymal organs from dead or slaughtered animals, lymph nodes, etc.),

conclusions of veterinary authorities, data collected in the course of independent research are used.

During the dissertation work, 45 guinea pigs and 513 cattle were used for experiments to determine the immunogenicity of brucellosis vaccines; more than 60,000 cattle immunized with various brucellosis vaccines in different regions of the Republic were used to evaluate the effectiveness of vaccines in production.

In the experiments carried out, the vaccine strain *B.abortus 19* and the BRUCON vaccine prepared at the LLP "ANTIGEN "SPE" for conjunctival immunization of animals against brucellosis" were used.

Determination of the epidemic situation for brucellosis in animals is carried out on the basis of reports and data from official veterinary authorities and the results of independent studies collected during business trips to the farms being inspected. For this purpose, the epidemiological situation of brucellosis in animals over the past 5-7 years are being studied in deeper level.

During the monitoring studies, the annual veterinary report and statistical materials and examination data of the Republican veterinary laboratory, the antiepidemiological detachment for 2014-2019 and scientific reports of KazSRUI were used. Epidemiological methods were used to analyze the number and dynamics of brucellosis-affected points, such as the analysis of long-term indicators, the creation of a graphical table, comparison of the number of registered unfavorable points from brucellosis of different animals by year, logical analysis of the results obtained.

Epizootological studies were carried out according to the methods proposed by Bakulov I.A., Dzhupina S.I., Avilov V.M.

To diagnose brucellosis, bacteriological and serological studies were carried out in accordance with the "Instructions for the diagnosis of brucellosis of animals" approved by the Ministry of Agriculture of the Republic of Kazakhstan. DCR analysis was carried out according to TC 9388-187-00494189-99 using the VRUCOM test system, and to confirm the relationship of the tested strains of brucella field isolates with S-type species, the classic version of DCR was used using the AMOS kit developed by Brickeretal.

DNA was isolated using Prelink Genomic DNA Kit s (Invitrogen), multiplex DCR and capillary electrophoresis (CE) were performed according to the algorithm with minor changes. The obtained data were analyzed using the BioNumerics7.5 software (AppliedMaths, Belgium). Statistical data processing was carried out according to the method of E.K. Merkur'yeva.

The main provisions of the dissertation, put on defense, are:

- The epidemic situation of animal brucellosis in the territory of the Republic of Kazakhstan in recent years and the effectiveness of specific measures to combat brucellosis;

- Results of a study of the genetic diversity of brucella using multilocus analysis of a variable number of tandem repeats (MLVA-16);

- The level of immunity in guinea pigs vaccinated with different doses and methods of the *B.abortus 19* vaccine against brucellosis.

- Post-vaccination dynamics of antibodies in calves vaccinated with different doses and methods of *B.abortus 19* vaccine;
- Epizootological effectiveness of the method of inoculation of the *B.abortus 19* vaccine into the conjunctiva in production conditions.
- Recommendations on carrying out special veterinary measures for the prevention of brucellosis of cattle on the territory of the Republic of Kazakhstan.

Description of the main results of the study.

In the republic in 2017-2019, the level of animal brucellosis morbidity was determined and zoning maps were compiled based on this indicator, allowing to visualize the distribution area of brucellosis of animals and possible risks of its spread, and they can also be used in the implementation of veterinary control over the development of infection.

For the first time, such extensive indicators as the spread of the epizootic process in brucellosis of animals in the territorial units of the Republic of Kazakhstan and their infection with brucellosis were studied. It can be used by veterinary specialists when planning and organizing measures to combat brucellosis.

The study of the genetic diversity of brucella is necessary for monitoring outbreaks of brucellosis or tracking the sources of infection of humans and animals in non-endemic areas of Kazakhstan using multilocus analysis of a variable number of tandem repeats (MLVA-16), brucella strains were identified. 2 isolates of *B. melitensis* isolated from WK were assigned to genotype 3, similar to the genotypes common in the southern regions of Kazakhstan, and 7 strains isolated from cattle were identified as *B. abortus* and grouped into 2 genotypes. The genotypes of strains 1 and 2 were genetically different from the strains found in Kazakhstan.

The effectiveness of vaccines used against bovine brucellosis in 2015-2019 in the Republic of Kazakhstan has been determined. The study of post-vaccination immunological reactions in guinea pigs with conjunctival vaccination showed that it disappears much earlier than with subcutaneous immunization. In the experiment, the intensity of immunity against brucellosis in guinea pigs 6 months after conjunctival vaccination was 90%.

When 80 billion microbial cells of this vaccine were vaccinated by the conjunctival method, antibodies were preserved in heifers up to 180 days, and in calves up to 150. At a vaccine dose of 5 billion mc, antibodies in heifers were preserved for 150 days, and in calves — for 90 days. Therefore, calves vaccinated by the conjunctival method at 4-6 months can be examined for brucellosis 4 months after immunization, and heifers immunized at 18-20 months after 6 months. This makes it possible to conduct an annual routine diagnostic examination of animals for brucellosis without hindrance.

In production experiments conducted in individual farms of Aktobe, West Kazakhstan and Karaganda regions, cattle immunized by the conjunctival method with the *B.abortus 19* vaccine against brucellosis were not detected during veterinary observation for 1.5-2 years, clinical signs of brucellosis were not

observed among animals. These data indicate the effectiveness of conjunctival administration of the *B.abortus 19* vaccine against bovine brucellosis.

Justification of novelty and importance of obtained results: The epidemiological situation of brucellosis of animals in the Republic of Kazakhstan in recent years has been determined, the effectiveness of specific measures to combat brucellosis has been analyzed, the importance of determining extensive (qualitative) indicators of the epidemic process during epidemiological monitoring of brucellosis of animals in Kazakhstan has been proved.

Multilocus analysis of a variable number of tandem repeats (MLVA-16) was proposed to identify brucella strains for source control in brucellosis-free areas.

The immunological efficacy (post-vaccination dynamics of antibodies, immunogenicity, etc.) of the *B.abortus 19* vaccine was determined when it was injected into the conjunctiva of animals, the optimal method of using the *B.abortus 19* vaccine for the prevention of outbreaks of brucellosis in farms was proposed.

Practical value of the work:

1. Epizootological maps of the territory of the Republic of Kazakhstan on the incidence of brucellosis in animals allow you to visualize the area of brucellosis and the possible risks of its spread.

2. Veterinarians can use detailed indicators of the epizootic process in brucellosis of animals, which are determined by the level of distribution in the territorial units of the Republic of Kazakhstan, when planning and organizing measures to combat brucellosis.

3. Multilocus analysis of a variable number of tandem repeats (MLVA-16) can be used to identify brucella strains, study the genetic diversity of brucella and track the sources of outbreaks among humans and animals.

4. During veterinary observation of cattle immunized with the *B.abortus 19* vaccine conjunctivally in production experiments conducted in individual regional farms for 1.5-2 years, animals with a positive result for brucellosis and clinical manifestations of brucellosis among animals were not detected. These data indicate the effectiveness of the *B.abortus 19* vaccine against bovine brucellosis by the conjunctival method.

5. It was found that 4-6-month-old calves immunized with *B. abortus 19* at a dose of 5 billion m.c. by the conjunctival method can be examined for brucellosis 4 months after vaccination, and 18-20-month-old heifers - after 6 months. This condition allows for unhindered annual routine diagnostic examination of animals for brucellosis.

Compliance with the main directions and development of scientific state programs. The dissertation work was carried out within the framework of scientific and technical support of veterinary health and food safety in the Republic of Kazakhstan for 2018-2020 (code 0.0870, state registration number 0118RK01221) - within the framework of the task "Ensuring veterinary and sanitary safety and epizootic well-being".

Research work in 2018-2020 was carried out at the Department of "Biological Safety" of the Kazakh National Agrarian Research University, at the SPE "Antigen", in the brucellosis laboratory of the Kazakh Scientific Research

Veterinary Institute LLP (KazSRUI), on the basis of branches of the SRUI and in individual epizootological units in various regions of the Republic of Kazakhstan

Description of the doctoral student's contribution to the preparation of each publication. The dissertation for the degree of doctor PhD academy of sciences, personally completed by the author, is a completed research work and meets the requirements of the Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan. The reliability of the research results of the dissertation work and the validity of experimental work are confirmed by photographic materials; research certificates.

Scope and structure of the dissertation. The materials of the dissertation are made on 106 pages and include: normative references, definitions, designations and abbreviations, introduction, literature review, independent research, discussion of research results, conclusion, practical recommendations, list of references.

The work is provided with 27 tables and 13 figures. The number of used domestic and foreign literature – 133.