

Improvement of allergic diagnostics of animals under the conditions of Uzbekistan

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Abstract. This article presents preliminary results of our own scientific and experimental studies of specificity, antigenic activity, safety, reactogenicity, and other properties of various allergic diagnostic tests made from strains of brucella with different species and virulent affiliation as the expected standards of industrial crops. As a result of the studies, Uzbek analogues of the standards of production strains of brucella strains intended for the manufacture of allergy diagnostics have been developed.

1. Introduction

Brucellosis (Brucellosis) - mainly a chronic disease of animals caused by bacteria of the genus "Brucella" which belongs to the 2nd group in terms of infection and is a very dangerous infectious and allergic disease for people. The specific clinical signs of animal brucellosis are characterized by massive abortions, infertility, decreased fetal viability and animal productivity.

At present, the epizootic and epidemiological situation of brucellosis in Uzbekistan is not entirely favorable, since it is determined by the presence of brucellosis among farm animals, which are the main causes of brucellosis in humans.

Sporadically, brucellosis is registered everywhere in the Republic. On average, the prevalence in the republic is among cattle (cows) - 0.01%, small cattle - 0.04%. This trend has persisted over the past 10 years.

Nowadays, the country's existing complex of organizational, economic, veterinary, sanitary and general preventive measures are expensive, time-consuming and not always effective because they are based on measures intended for large livestock farms complexes, etc. In Uzbekistan, the method of introducing livestock is different in the number of livestock, mainly it is found in private farmsteads of dekhkans and farms, where grazing is predominant.

In the economically developed United States, Germany, the Netherlands, Denmark, China, Russia, South Korea, Turkey, and other countries, animal brucellosis also continues to be registered with different levels of intensity, although these countries are mainly farmers and farms, however, it is mainly found in complexes where stall content prevails.

Therefore, brucellosis in all countries is perceived as a global problem where control and prevention are the basis of the fight against this disease.

For this purpose, in Uzbekistan and around the world it is recommended to widely use serological diagnostic methods: RBP - rose bengal test, KR - ring reaction with milk, RA - agglutination reaction, RSK - complement fixation reaction, RDSK - long-term complement fixation reaction (for infectious sheep epididymitis), ELISA - enzyme immunoassay, PCR - polymerase chain reaction, and along with



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them - allergic diagnostics. All of these methods are recommended by the FAO / WHO and OIE for the diagnosis of brucellosis. (Terrestrial Animal Health Code, 2018).

Recently, the vaccination of animals has become the basis of the strategy for combating brucellosis in many countries of the world. This method has proven itself in many European countries, where the cases of the isolation of brucellosis animals are minimized and in some countries it was completely possible to eliminate this disease among cattle and small cattle (USA, France, Germany, Holland, etc.).

Vaccination of animals against brucellosis requires a strict attitude to the issues of accounting, livestock migration, compensation to animal owners in case of detection of cattle infected with brucellosis, timely diagnostic tests, the competence of specialist laboratories and other issues.

In Uzbekistan, over the past 20 years, an allergic diagnosis of brucellosis has not been used. Although this method is most acceptable as an additional test for farms where vaccination is not used and is very convenient for distant cattle breeding.

The allergic method is convenient, simple and reliable in performance and affordable for practical veterinary medicine.

A lot of research has been done in the world on the creation and use of allergens. One of the most deserved is the creation in 1968 by E.S. Orlov and A.N.Kasyanovs from a non-agglutinogenic strain - brucellin VIEV, which is still used in the diagnosis of brucellosis in sheep, goats, pigs and deer. It is known that the allergic state of a sick animal occurs after the formation of specific antibodies in the body and persists for a long time, that is, up to 16 months (P.P. Samoilov). Although in the literature there are other works on brucellosis allergen intended for the diagnosis of brucellosis.

Thus, the issues of studying allergic diagnostics are relevant and promising.

In Uzbekistan, allergic diagnostics is included in the general complex of a scientifically based system for the diagnosis of brucellosis used in both prophylactic and health-improving measures for brucellosis, goats and pigs. However, due to the fact that these allergens are not produced in the country and there are no commercial allergens, allergy testing is not conducted.

The aim of the research was the following:

- Comparative study of the cultural, morphological, biochemical, virulent and other properties of the local epizootic and selection vaccine strains of Br.abortus 104M UZ, 2017/1 UZ and Br.melitenzis Rev-1 UZ, 9 UZ from the collection of the NIIV museum in comparison with the reference strains Br.abortus 544, Br.melitenzis 16M and Br.suis 1330 with the aim of selecting the most suitable for the manufacture of anti-brucellosis allergens.
- from the selected brucella strains to make experimental microseries of anti-brucellosis allergens using different methods and conduct a comparative study of the activity, sensitivity, sensitizing properties, safety, reactogenicity, agglutinogenicity in experiments on laboratory animals (guinea pigs, white mice, sheep).

Materials and methods. All selected strains of Br.abortus 104M UZ, 2017/1 UZ and Br.melitenzis Rev-1 UZ, 9 UZ, as well as three reference Br.abortus 544, Br.melitenzis Novochoerkassk-102 (VGNKI) and Br.suis 1330 adapted to the nutrient medium MPPGGA (meat-peptone hepatic glucose-glycerin agar) by three reseeded of isolated colonies and growing in an incubator for 72 hours. After obtaining pure cultures, each strain was seeded with 5 matry flasks containing MPPGBH on medium with MPPHGB (meat-peptone hepatic glucose-glycerin broth). All flasks with a nutrient medium were placed in a thermostat for further incubation at 37 ° C. The terms of incubation were different and ranged from 10 to 20 days with the aim of maximum accumulation of waste products.

After a certain time, a nutrient medium containing brucella and their decay products was taken from the kolbot and subjected to various treatment methods (heating to 95-1000C for 1-2 hours, heating in the same mode in the presence of 1% phenolized saline solution, freezing and thawing after inactivation at 95-1000C and other methods.). The growth characteristic of brucella was monitored visually. The obtained concentrates of allergens manufactured by different methods were centrifuged at 5 thousand rpm 1 fraction and 10 thousand rpm. in an hour. The precipitated bacterial mass was not used further in

the production of allergens. The rest of the allergen concentrates containing brucellin extracts were preserved with phenol until a final dilution of 0.3% was obtained. The obtained allergen samples with 1% phenol were resuspended with saline to obtain a final phenol concentration of 0.3% in the extract. All obtained samples of allergens were placed in the refrigerator for 15-20 days to stabilize.

After stabilization, a quantitative determination of the protein was carried out by the nephelometric method.

The purity of the prepared allergens was checked by microscopic methods using Gram stain and Kozlovsky stain.

The purity of growth was determined by seeding on MPPGGA and MPPGB. The pH density was determined by potentiometric methods and the pH was adjusted to 7.0-7.2 by alkalization with a 4% sodium hydroxide solution.

Research results. The cultural, morphological biochemical, and other properties of the local epizootic and selection vaccine strains of *Br.abortus* 104M UZ, 2017/1 UZ and *Br.melitenzis* Rev-1 UZ, 9 UZ were tested in comparison with the reference cultures of *Br.abortus* 544, *Br.melitenzis* Novochoerkassk-102 (VGSKI) and *Br.suis* 1330 in accordance with the methods recommended by the FAO / WHO for working with brucella cultures.

The affiliation of brucella cultures of one kind or another was studied by the ability to form hydrogen sulfide (H₂S) with lead acetic acid, the tinctorial properties of sensitivity to basic fuchsin and thionine when it was contained in various concentrations in a nutrient medium (1: 25000, 1: 50000, 1: 100000) The virulent properties of the studied cultures will be evaluated by growth and sensitivity to various concentrations of penicillin in the nutrient medium (0.5, 5, 10, 50 IU / ml) and erythritol.

It was found that all the studied strains have characteristic species properties according to these indicators. The strain *Br. abortus* 2017/1 actively produced hydrogen sulfide, and the average total indicators of the size of the blackening of the indicator paper was more than 12 mm (counting was carried out every two days).

Strains *Br. abortus*104M UZ and *Br. melitenzis* Rev-1 UZ, 9 UZ did not produce hydrogen sulfide. For the strain *Br. abortus* 104M UZ, this trait is characteristic and serves as a marker that allows you to identify it as a biotype -6. The results obtained using the reference strains *Br. abortus* 544, *Br. melitenzis* Novochoerkassk-102 (VGSKI) and *Br. suis* 1330 also indicate their compliance with the passport data.

When studying the virulent properties of cultures in preliminary tests for growth and sensitivity to various concentrations of penicillin in a nutrient medium (0.5, 5, 10, 50 IU / ml) and erythritol, it was found that cultures of field strains of *Br.abortus* 2017/1 *Br.melitenzis* 9 UZ are not sensitive to this antibiotic at its concentration in MPPGA 50 U / ml, while vaccine strains of the strain *Br.abortus* 104M UZ and *Br.melitenzis* Rev-1 UZ, to concentrations of 5 and 10 U / ml. were sensitive and did not grow on these media. All cultures of reference strains (*Br. Abortus* 544, *Br. Melitenzis* Novochoerkassk-102, and *Br. Suis* 1330) were also not sensitive to penicillin and grew well when it was kept in MPPGA at a concentration of 50 U / ml. On a medium with erythritol (1: 1000), all cultures of the studied field strains grew well in a sowing dose of 100 microns. cells / ml Reference strains of *Br. abortus* 544, *br. Melitenzis* Novochoerkassk-102, and *Br. suis* 1330 were also not sensitive to erythritol at a dose of 100 µl / ml i.e. observed their rapid growth.

The vaccine strains of *Br.abortus* 104M UZ and *Br.melitenzis* Rev-1 UZ did not grow on the medium containing erythritol when sowing 100 µl, but single colonies were observed when the strain of *Br Melitenzis* Rev-1 was inoculated with a dose of 1 million µl. class This indicates its higher virulence compared with the culture of the species *Br.abortus* 104M UZ. Thus, despite the long-term storage of museum and epizootic strains of brucella at MPPHA and the periodicity of their reseeded for a long time, they all retained their main species and type cultural morphological, biochemical, virulent and other properties and remain stable and epizootically significant.

The agglutinable properties of the prepared antigens from each strain separately were studied in the classical agglutination reaction to the limit titer. It was found that the titers of detectable antibodies with antigens from field strains were lower than the titers in the same sera with antigens from vaccine strains of brucella.

Results of agglutinable, virulent and biochemical properties of epizootic strains of *Br. abortus* 1 / 2017UZ and *Br. melitensis* No. 9 allows using them as domestic reference preparations along with commercial reference strains of *Br. Abortus* 544 (Weybridge), *Br. Melitensis* Novocherkassk-102 (VGNKI).

The stability, variety and degree of dissociation of colonies of epizootic cultures of brucella was studied using the thermoagglutination reaction, the use of acryllavine, S and R positive sera and the White-Wilson method using a crystal violet in a working dilution of 1: 2000. At the same time, the stability of breeding colonies with clearly expressed S and R brucella forms were studied by passage multiplicities and storage periods on nutrient media.

All selected strains of the species *Br.abortus* 104M UZ, 2017/1 UZ and *Br.melitenzis* Rev-1 UZ, 9 UZ, according to the results of preliminary studies (2018-2020) were deposited in a unique collection of animal microorganisms of the NIIV with passports in the established form, included to the depository list and intended for the manufacture and study of them as domestic anti-brucellosis vaccines and diagnostics.

The protein content in the extracts of the experimental series ranged from 18-27 mg%.

The safety test was carried out on healthy white mice weighing 18-22 g. Three heads of white mice were taken for each microseries. Allergens were injected into the back area in a volume of 0.5 ml and were observed for ten days. During the observation period, all experimental mice remained alive and deviations from physiological norms were not observed.

The specificity of the tested allergens was studied on guinea pigs weighing 300-350 g. By which the drug was injected into the depilated area of the lateral torso intradermally in a volume of 0.1 ml. Compared to commercial brucellin containing 200 activity units (EA). As a result of the inactivation of the experimental microseries of allergens made by different methods, after 24-48 hours, the characteristic diffuse inflammatory processes, redness, and significant swelling were not established, which testified to the specificity of all tested allergen samples.

The specificity test and the absence of agglutinogenic properties of the experimental allergen microseries on healthy 15 heads of Karakul sheep when introduced under the skin in a dose of 0.5 ml (palpebrally), taking into account the results of allergic reactions after 24 hours (primary) and 48 hours (finally), also indicates the high specificity of the tested brucellodiagnostics. The study of agglutinogenic properties 10-15-20 days after the introduction of allergens to these 15 sheep heads indicates the absence of anti-brucellosis antibodies in the blood during the study of blood clots in the Rose-Bengal test and the agglutination reaction with brucellosis antigens.

The reactogenic properties of allergens were determined during a 5-day control, at which the following results were obtained: the sheep were kept together with free watering and feeding, while the average daily body temperature was $\sum X-39.0^{\circ}\text{C}$ using an allergen made from strain 104MUZ. When using allergens from strains Rev-1UZ, 1 / 2017UZ, 9UZ, no changes in the physiological state of the sheep were also observed, and the average body temperature was $-X-39.80^{\circ}\text{C}$, $\sum X-39.40^{\circ}\text{C}$ and $\sum X-39$, respectively. 60°C .

Currently, studies on the properties of allergens made from strains of brucella with various species and virulent accessories and made by various methods are ongoing.

2. Conclusion

As a result of the studies, Uzbek analogues of the standards of production strains of brucella strains intended for the manufacture of allergy diagnostics have been developed.

It has been established that all microseries of prototypes of antigens produced from local strains *Br. abortus* 104MUZ, 1/2017UZ and *Br. melitenzis* Rev-1UZ and 9UZ have sufficient specificity, safety, arereactogenicity against anti-brucellosis allergic diagnostics.

It was established that all tested allergens from the strains *Br.abortus* 104MUZ, 1 / 2017UZ and *Br.melitenzis* Rev-1UZ and 9UZ did not cause the production of specific anti-brucellosis antibodies detected in the Rose-Bengal test and agglutination reaction with brucellosis antigens.

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