Some issues related to the study of brucellosis in Uzbekistan

A D Ulugmuradov and M A Uzimurodov
Research Institute of Veterinary Medicine, Samarkand, Uzbekistan

E-mail: nivi@vetgov.uz

Abstract. This article provides some points on the fight against brucellosis in Uzbekistan as well as independent research on epizootology, chronology, characterization of anti-brucellosis vaccines and the properties of brucella strains with different species and virulence. The research results allow us to replenish the collection of standards of industrial crops of brucella that are necessary for the development of domestic production of anti-brucellosis vaccines and diagnosticums, as well as create national reference strains of brucella designed to control their quality. Knowledge of the properties of epizootic strains of brucella circulating in a particular territory allows us to classify farms and assess the degree of tension of epizootic and epidemic processes, to establish the fact of migration of brucella from one animal species to another, to identify pathways of pathogen distribution, and the choice of tactics for conducting antiepizootic measures. The presented comparative characteristic of anti-brucellosis vaccines enables specialists to choose the most effective and acceptable when carrying out measures for the prevention and elimination of this disease.

1. Introduction

The primary tasks that any government and state set are to increase the production of livestock products since they are of great strategic importance related to food safety.

In turn, the safety of livestock products directly depends on the provision by the veterinary service of the epizootic well-being of livestock for infectious diseases and, in particular, for brucellosis.

Brucellosis causing enormous damage to animal husbandry due to abortion, lack of offspring, stillbirth, barrenness, reduced productivity of sick animals, increased costs of activities associated with their recovery and prevention is of great economic importance.

In Uzbekistan, with the transition to a market economy, the introduction of livestock production has fundamentally changed. At present, about 96% of cattle and 94% of small cattle in Uzbekistan are located in the introduction of private farms and citizens. With the increase in the number of farms and private farmsteads the danger of the spread of brucellosis has sharply increased.

Nowadays, horses, pigs and camels in the epizootology of brucellosis spread in the republic are not significant due to their insignificant number and the lack of evidence of brucellosis among them. However, recently the number of farms engaged in camel breeding, horse breeding and goat breeding has been increasing.

Animal brucellosis is currently a global problem on a global scale, both for medicine and veterinary medicine.

The complete elimination of brucellosis is almost impossible. What is necessary is the complete destruction (disappearance) of the biological species of the pathogen, in this case brucella. Basically, this is impossible since brucellosis has a species diversity, i.e. there are main pathogens of brucellosis...
parasitizing on certain types of animals. So, on cattle - the species Br.abortus, on sheep and goats - Br.melitensis, on pigs - Br.suis, etc. Most species of brucella migrate from one animal species to another and thereby complicate the epizootological situation. Therefore, even if we remove the source of the pathogen and carry out all veterinary and sanitary measures in full, anyway, this regional territory will have an unstable state.

In this regard, studying the pathogen epizootology, biotypic affiliation; improving the means, methods of diagnosis and specific prophylaxis; searching for new highly effective ways to combat brucellosis are urgent tasks of veterinary science and practice not only in Uzbekistan but also in other countries.

2. Independent research

Brief epizootology of brucellosis. Animal brucellosis continues to be recorded with different intensities in the USA, Germany, Holland, Denmark, France, China, Russia, South Korea and other developed countries.

Brucellosis caused by Br.abortus is now officially considered eradicated in Canada, Australia, Japan and Northern Europe.

In the USA and Northern European countries that have made significant progress in controlling brucellosis for the past 50 years they used the strategy of compulsory vaccination of livestock in the beginning and, gradually, as the incidence among animals decreased, instead of vaccination the policy was “detection of brucellosis in animals and immediately slaughter” (test slaughter) which made it possible to practically eradicate brucellosis among farm animals. An important role was played by the state financial compensation to farmers and owners for the slaughtered animal.

The most endemic regions for brucellosis are the countries of the Mediterranean and the Middle East. According to recent publications a high incidence of brucellosis and its growth is noted in China, in almost all countries of South / Southeast Asia. In Central Asia the most endemic are Kyrgyzstan and Azerbaijan.

In Uzbekistan, brucellosis is sporadically recorded everywhere. According to our data the average prevalence in the republic is approximately: among cattle (cows) - 0.01% and among small cattle - 0.04% (These approximate figures have been preserved over the past 3 years). This relative prosperity for brucellosis can be maintained due to the fact that there is no concentration of a large number of livestock in business and private farms, therefore sanitary cleaning in these categories of farms gives its positive results. Farms with large livestock of 500, 1000 or more and that of often graze on seasonal pastures remain problematic. In such categories of farms the problem of brucellosis is associated mainly with cattle regrouping and untimely serodiagnosis.

Under these conditions the primary task facing veterinary specialists was to protect the health of the population from brucellosis, i.e. its social significance has increased.

Therefore, the main methods for the control and prevention of brucellosis aimed at artificially reducing the incidence of animals to a relatively low level when it occurs or spread within areas are recording and registering, chipping and compensation issues for animal owners when removing livestock. Since these issues are one of the forms of solving the problem of brucellosis and other zoonotic animal diseases in Uzbekistan they are considered at the government level.

The control of brucella species and biovars today in Uzbekistan also allows to classify foci, to carry out their accounting and registration. This allows us to assess the degree of tension of epizootic and epidemic processes, to establish the fact of migration of brucella from one animal species to another, to identify the pathway of the pathogen, to choose the tactics for antiepizootic measures.

Currently, it is customary in the country to determine the degree of disadvantage of herds and farms taking into account the level of distribution of brucellosis among animals:

- limited - in case of disease within one year, up to 2% of the animals from the presence in the herd (farm);
- significant - with the disease, up to 10% of the animals from the presence in the herd (farm);
mass - with a disease of more than 10% of the animals from the presence in the herd (farm).

Based on this, control over the well-being of farms is carried out with the help of:

- annual planned diagnosis of livestock, including young animals older than 2-3 months. (RBP, KR, RA, IFA and, if necessary, RSK);
- the presence of a veterinary certificate or certificate for new animals;
- quarantine newly arrived animals, within 30 days period they must conduct a physical examination and serological tests;
- the issuance of each prosperous farm a special certificate.

2.1. Chronology of the fight against brucellosis in Uzbekistan:

- 1925-1952 - campaign for checking and slaughtering cattle, sheep and goats.
- 1953 - CDP vaccination campaign along with screening and slaughter.
- 1991 - privatization of livestock farms, the creation of small livestock farms and the expansion of private farms. (Disruption of surveillance, increase in prevalence among animals).
- 1996 - change in the vaccination campaign of the cc: full dose for 3-6 month old calves, revaccination of heifers with small doses of the vaccine from strain 19.
- 1998-2007 - for the first time, vaccination of sheep and goats with a vaccine from a strain - 19.
- 2007- application of Rev-1 vaccines for sheep and goats on selected farms.

This system of anti-brucellosis measures used in Uzbekistan with the use of vaccines in certain regions of the country significantly improved the epizootic situation in brucellosis of cattle and small cattle. Despite this, the search for new vaccines is ongoing and schemes for their use are being improved.

2.2. Brief description of anti-brucellosis vaccines:

Currently recognized and widely used are vaccines from strains of Br.abortus 19 and Br.melitensis Rev-1.

The vaccine from the strain Br.abortus 19, known since 1934 and is the most highly effective vaccine among all anti-brucellosis vaccines in the world, it is harmless, sharply reduces the number of abortions and positively reacting sick animals. The vaccine does not reduce milk yield, does not migrate from vaccinated to unvaccinated animals. The vaccine prevents animals from naturally becoming infected, although immunity may be insufficient in herds with the acute course of brucellosis.

Immunity tension is about 71.4–87.5% for 338 days. (Period of own observations). Revaccination 12 months later, including a small dose after immunization, enhances the immune state of the body by 12.5–29.6%.

The main disadvantage of the vaccine is that the blood serum of the vaccinated animals retains agglutinins and complement-binding antibodies for a long time, which makes it impossible to distinguish animals vaccinated with brucellosis and to give an objective assessment of the epizootic situation in the country. At the same time, when a small dose of the vaccine from strain 19 is revaccinated the immunity of the same strength is created in cows and heifers as with a standard dose, it does not interfere with the normal course of pregnancy and cause short-term seropositivity in vaccinated animals. In Uzbekistan, this vaccine has been widely used for many years and its application schemes (including a small dose) have been developed.

In 1953, American scientists Elberg and Herzberg selected a streptomycin-dependent mutant from a virulent culture of strain Br.melitensis 6056. Then, on its basis, a reverse mutant was obtained, which grew without streptomycin. The new variant they obtained was called Br.melitensis Rev-1. A vaccine made from this strain is the most immunogenic of all anti-brucellosis vaccines in the world. This vaccine for immunization of sheep and goats is used by all countries of the world where there is brucellosis of small cattle. A single vaccination of the rodents and goats with the vaccine from the strain Rev-1
provides intense and long-lasting immunity (observation period is 5 years), and therefore there is no need to revaccinate adult animals (Ulasevich P.S. et al., 1974, 1975; Ivanov N M.M. et al., 1981, 1988; Alton GG, 1987). According to other researchers, in farms where a single vaccination was carried out with a vaccine from the strain Rev-1, cases of brucellosis in animals were observed after 2.5 - 3 years. Reimmunization of sheep with the vaccine from Rev-1 strain helps to preserve post-vaccination antibodies for a long time, which makes it difficult to differentiate animals vaccinated and patients with brucellosis (Sultonov A.A., Ivanov N.P. et al., Yagudin R.G. and other).

The disadvantage of this vaccine is its high residual virulence, agglutinogenicity and not rarely abortogenicity. (own research).

Noteworthy is the vaccine from the strain Br.abortus 104M selected in 1950 by Soviet scientists from a culture isolated from an aborted cow fetus.

The strain Br.abortus 104M is in a stable S-form, is weakly virulent, has pronounced antigenic and higher immunogenic properties than strain 19. It is easy to control and differentiate from other strains since it has only its own marker (does not produce H2S), non-abortogenous and does not migrate from vaccinated animals to unvaccinated, less antigenic than strain 19.

The vaccine from strain 104M passed a wide test with a positive result in production conditions on cattle and deer (Shumilov K.V., Klimanov A.I., Mikhailov N.A., Kalmykov V.V., Sklyarov O.D. and other).

From the latest new means of specific prophylaxis is known the vaccine from the strain Br.abortus RB51 obtained by Schurig and officially recognized in 1996 in the United States and in 2002 by the European Union: an assessment by various authors interprets it differently, many indicate weak immunogenicity and tension of the immune system.

In Uzbekistan, over the years, domestic vaccines have been developed from the strain Br.melitensis Nevsky-12 (R-form) which was a derivative of the Nevsky-13 (RS-form) and Nevsky-14 (S-form) vaccines (Abidzhanov M., Vafakulov A.Kh., Grinko V.K., Yaraev R.G. et al.). The most promising and more studied is the vaccine from strain Nevsky-13. The disadvantage of this live vaccine along with the lack of immunity is the quality control of its manufacture in connection with the variability of this strain (instability). The adjuvant vaccine from this strain turned out to be more preferable compared to live, however, it had more pronounced reactogenic properties. (Shumilov K.V., Klimanov A.I., Grinko V.K. et al.). The vaccine from Nevsky-14 strain has not been adequately studied although it has been fully studied as a single brucellosis antigen for RA, RSK, RDSK, and the possibility of its use as a diagnosticum has been established provided that its activity is standardized for the international agglutinating serum anti-brucella abortus. (Shumilov K.V., Klimanov A.I., Ruzimurodov M.A.).

Thus, knowing the polytropy of the causative agent of brucellosis the multiplicity of factors for its transmission from a healthy patient, the route of infection, etc., we conducted research on the basis of the Research Institute of Virology (RIV) to restore the collection of standards for production strains of brucella.

Based on the results of studying the archival materials of the brucella collection museum strains of Br.abortus 104M, 19, B.B. melitensis Rev-1 as well as 10 epizootic strains of the abortus type were selected (51, 92,114, 126, 207, 278, 337, 338, 604, 1 / 2017UZ) and melitensis (4, 30, 193, 9, 81, 653, 88, 84, 452, 95 UZ) to study their cultural morphological, biochemical, tinctorial, virulent and other properties. The main purpose of these studies was to establish the possibility of using individual strains from the museum's collection as reference preparations and standards for the production of domestic vaccines and diagnosticums.

The results of studies of the properties of 10 epizootic cultures of the abortus and melitensis species in comparison with the reference strains of Br.abortus 544, Br. suis 1330, Br.melitensis Novocherkassk-102 (All-Russian State Center for Quality and Standardization of Medicines for Animals and Feed) showed that despite the length of reseeding and storage all strains retained good growth properties and do not have pronounced persistent signs of dissociation.

So, in the sample with acriflafin (1: 1000), with R and S brucellosis sera (1:10), and the White-Wilson method signs of dissociation were not detected in the colonies of the primary generations; later, in
the 3-4 generation cultures these signs were absent. The results of the study in tests with penicillin (0.5; 5; 10; 50 U/ml) showed that all selected cultures of field strains are not sensitive to this antibiotic at its concentration in MPPGA 50 U/ml. Whereas the vaccine strain of Br. Abortus 19 was sensitive to a concentration of 0.5 U/ml and the strains of Br. Abortus 104M and Br. Melitensis Rev-1 to a concentration of 5 and 10 Units/ml respectively. All cultures of reference strains (Br.abortus 544, Br.melitensis Novocherkassk-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. The reference strains of Br.abortus 544, Br. Melitensis Novocherkassk-102, and Br.suis 1330 were also not sensitive to erythritol at a dose of 100 μl/ml i.e. observed their rapid growth. Vaccine strains of Br.abortus 19, 104M and Br. (microbial cells), but single colonies were noted when plating the strain Br. melitensis Rev-1 with a dose of 1 million microns. class This indicates its higher virulence compared with cultures of the species Br. Abortus 19 and 104M.

Thus, despite the long-term storage of museum epizootic strains of brucella at MPHGGA (meat-peptone hepatic glucose-glycerin agar) and the periodicity of their reseeding for over 65 years, 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. However, this difference in credits was not statistically significant.

The results of agglutinable, virulent and biochemical properties of epizootic strains of Br.abortus N 92, 126, 207, 1/2017 UZ and Br.melitensis N 4, 9, 633 allow them to be used as domestic reference drugs instead of the commercial reference strain Br.abortus-544 (Weybridge).

Domestic standards for vaccine strains of the type Br.abortus 19 UZ, 104M UZ and Br.melitensis Rev-1 UZ were selected from individual cell clones of museum vaccine cultures 19, 104M, Rev-1 and passports in the established form were compiled and deposited in the "Unique Collection of Animal Microorganisms of RIV (the Research Institute of Virology) ". Studies of the vaccine properties of these strains are ongoing.

3. Conclusion
In Uzbekistan, taking into account animal husbandry, the implementation of recreational activities should be based on seromonitoring and vaccination. Based on the results of seromonitoring, establish farms and territories where it is advisable to use vaccines from strains 19 and Rev-1. In cases of the prevalence of brucellosis among cattle below 2%, apply the strategy of "identification and destruction" that will be economically justified.

It has been established that despite the long-term storage of museum, epizootic strains of brucella at MPHGGA and the periodicity of their reseeding for over 65 years, they all retained their main species and type properties and remained stable and epizootically significant.

The results of a study of the agglutinable, virulent and biochemical properties of epizootic strains of Br.abortus N 92, 126, 207, 1 /2017UZ and Br.melitensis N 4, 9, 633 indicate the possibility of their use as domestic reference drugs instead of a commercial reference strain of Br. abortus-544 (Weybridge).

Based. on the data obtained for the strains of Br.abortus 19 UZ biotype-1, Br.abortus 104M-variant UZ biotype-6 and Br.melitensis Rev-1-variant UZ biotype-1, passports are drawn up in the established form on the basis of which they included in the depository of the "Unique Collection of Animal Microorganisms of RIV".

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