

REVIEW

of the dissertation of / ngirbai A. Bakytkali on the topic: "Using multiprotein recombinant antigen for serological diagnosis of brucellosis", submitted for the academic degree of Doctor of Philosophy (PhD),
Specialty 6D120I00 - "Veterinary Medicine"

Brucellosis is one of the most widespread zoonoses in the world, and in a number of countries, including the Republic of Kazakhstan (RK), the disease has become endemic. The main link in anti-epizootic measures is the timely detection of productive ruminants with brucellosis. Serological tests, such as Agglutination Test, Complement Fixation Test and Rose-Bengal Test, developed at the beginning and / or in the second half of the last century and used in the diagnosis of infection, do not reliably detect infected cattle and/or sheep, which is the main reason for the circulation of the pathogen in the environment.

These traditional tests are based on the detection of antibodies against the pathogen cell wall lipopolysaccharides, which does not exclude false positive results due to cross-reactions with related bacteria. Commercial ELISA kits available on the veterinary market are also based on the use of *Brucella* spp. polysaccharide antigens. Therefore, the development of tests based on the use of a specific antigen and allowing to avoid cross-reactions with closely related bacteria is one of the urgent tasks of veterinary science not only in the RK, but also in other countries endemic for brucellosis.

Among the nonpolysaccharide *Brucella* cell components that can minimize cross-reactions are proteins, including outer membrane proteins (Omps). Advances in genetic engineering have also eliminated difficulties in antigen preparation and the biohazard risks associated with cell culture and opened up new prospects for the study of *Brucella* recombinant OmPs (rOmps) diagnostic value. A review of the world literature in the field of improving the diagnosis of brucellosis shows that the use of a single romps provides the specificity for indirect ELISA but significantly reduces its sensitivity. The combined use of *Brucella* rOmps imparted a higher sensitivity to the assay in testing brucellosis positive blood serum samples. However, the use of several single proteins as an antigen leads to an increase in the cost of diagnostic tests. Thus, a chimeric (fusion) antigen comprising the most diagnostically important regions of several proteins and synthesized by a single producer strain would provide high accuracy for the analysis and the relative cheapness of a diagnostic kit.

The dissertation (thesis) of B. Ingirbay aims to obtain *Brucella* spp. recombinant chimeric OmPs - producing strains of *E. coli*, and to study the value of target products as antigens in ELISA for serological diagnosis of cattle and sheep brucellosis. Three types of antigen, each comprising the immunodominant regions of two *Brucella* OmPs, were successfully expressed in *E. coli* BL21 (DE3) cells using the PET28 plasmids and their efficacy was assessed in an attempt to increase the sensitivity of the i-ELISA for serological diagnosis of bovine and sheep brucellosis. These chimeric proteins, designated rOmp19 + 25, rompl9 +

31, and rOmp25 + 31 were composed of active serological parts of *Brucella* spp. Omps with molecular weights of 19 kDa, 25 kDa, and 31 kDa. The results showed that chimeric rOmp 19 + 31 has pronounced immunoreactive properties compared to other proteins used, and might be used in the serological diagnosis of animal brucellosis.

The scientific novelty of the dissertation work lies in the fact that for the first time a prokaryotic producer strain synthesizing a chimeric protein consisting of immunodominant fragments of *Brucella* Omps with molecular weights of 19 kDa and 31 kDa was obtained, and this chimeric antigen was used in the development of a sensitive and specific ELISA for serological testing of cattle and sheep. The strain-producer of the chimeric protein is recognized as an invention and protected by a patent of the RK.

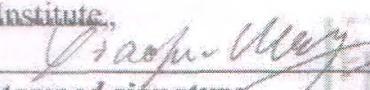
The thesis is a complete independent research work containing a new solution to one of the important tasks of veterinary science and has practical value. The novelty of the results allows further study on the commercialization of the experimental ELISA kit.

In the course of implementing the tasks of the dissertation, the academic degree applicant has sufficiently mastered modern methodology and methods of scientific analysis, acquired the skills to conduct in-depth scientific research in the field of immunology and genetic engineering.

The results of the dissertation work were presented at three international conferences, and were also reported at the First China-Kazakhstan Veterinary Medicine Science and Technology Postgraduate Academic Forum (May 28, 2022). The main results of the thesis were published in three articles included in the Scopus database.

I believe that Ingirbai K. Baktykali, an applicant for the academic degree of PhD, deserves to be awarded the degree of Doctor of Philosophy (PhD) in the specialty 6D120100 - Veterinary Medicine.

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Взыскано согласно, ст.30 п.2
и ст.30-1 Закона РК «О нотариате».

Нотариус _____

Жанар

09-11-2022



Настоящий документ был переведен компетентным переводчиком ИП «Агентство переводов «STAR Translations»» Жеренкеновой Мадиной Ойратовной



ET7400502221109135110S985700
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