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STUDY OF THE IMMUNOREACTIVITY OF *BRUCELLA SPP.* RECOMBINANT FUSION PROTEINS

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Brucellosis is a worldwide zoonotic disease caused by bacteria of the genus *Brucella*. Brucellosis is transmitted to humans from domestic animals. Basically, the disease affects the reproductive system of domestic animals, causing abortion and infertility [1].

In this regard, the question arises of the use of specialized tests aimed at detecting brucellosis. Diagnostic tests that allow detecting this pathogen and distinguishing animals infected with *Brucella* from vaccinated or infected with other microorganisms, often having cross-reactions with *Brucella*, are needed.

The enzyme-linked immunosorbent assay (ELISA) attracts attention in conducting serological reactions for the detection of samples with *Brucella*. Manufacturers of diagnostic kits prefer polysaccharide components of bacteria because of their ease of obtaining. When ELISA is based on *Brucella's* whole cells or lipopolysaccharides, false positive results are inevitable [2]. Therefore, other antigens, that can solve the problem of incorrect results, are needed. Among the components of the *Brucella's* cell wall, protein components are the most promising in the development of diagnostic tests [3].

A number of proteins of the outer and inner membrane (cytoplasmic, ribosomal and periplasmic antigens) were characterized. It was proved that periplasmic proteins are less antigenic than the outer membrane ones, because they are localized between peptidoglycan and the inner membrane of the *Brucella* cell wall. Antibodies of the cytoplasmic proteins of *Brucella* are useful only for the diagnosis of human brucellosis. The distinctive properties of the outer membrane of *Brucella* are considered critical for virulence [3, 4].

At the present stage of the development of genetic engineering, one of the effective approaches to obtaining *Brucella*-specific proteins is the creation of strains producing recombinant proteins of the pathogen [5]. Of great practical interest in the development of an enzyme-linked immunosorbent assay is the use of a combination of more than one recombinant protein in order to increase its sensitivity [1].

The aim of this work was to study the immunoreactivity of fusion recombinant proteins of the outer membrane of *Brucella spp*.

Three groups of outbred white mice were used. 10 heads of each group were immunized with fusion recombinant proteins OMP19 + OMP25, OMP19 + OMP31 and / or OMP25 + pOMP31. Laboratory animals were immunized twice with an interval of 14 days. Serum samples were taken on the 28th day of immunization from the tail vein.

To determine the optimal concentration of the recombinant protein required to cover the wells of a 96-well plate, blood sera was taken from 4 cows (№1,2,3,4) vaccinated against brucellosis of the *Brucella abortus* strain, 1 cow experimentally infected with brucellosis (№5), and 1 breeding bull (№6), seronegative for brucellosis by classical reactions. The reaction was considered positive if the optical density (OD) of a well with blood serum from an animal vaccinated or infected with brucellosis exceeded the extinction values of the control well by two or more times. The results of an indirect enzyme-linked immunosorbent assay are shown in Table 1.

Table 1 – Determination of the optimal concentration of the fusion recombinant protein Omp25-Omp19 for the sensitization of the solid phase ELISA

Antigen concentration used to coat the wells of ELISA plates												
	1 μg / ml			5 μg / ml			8 μg / ml			10 μg / ml		
Antibody titer / animal number	1	3	5	1	3	5	1	3	5	1	3	5
Optical density (OD) values of the reaction liquid, 492 nm												
1: 100	0.842	0.756	1.933	0.833	**1.135**	2.089	**1.304**	0.897	2.589	1.670	1.561	2.808
1: 200	0.645	0.666	1.967	**1.139**	0.842	2.016	0.915	**1.088**	2.625	**1.319**	**1.262**	2.853
1: 400	**0.598**	**0.420**	1,564	0.822	0.642	1.698	0.737	0.767	2.225	0.904	0.899	2.489
1: 800	0.323	0.395	1.176	0.442	0.445	1.400	0.485	0.508	1.231	0.639	0.614	1.608
Antibody titer / animal number	2	4	6	2	4	6	2	4	6	2	4	6
Optical density (OD) values of the reaction liquid, 492 nm												

1: 100	0.972	1.190	0.283	1,216	1.198	0.557	0.374	1.529	0.672	1,746	1.714	0.691
1: 200	0.689	0.990	0.210	0.850	1.220	0.477	**1.130**	**1.223**	0.507	1.395	1.775	0.530
1: 400	0.822	0.545	0.173	**1.073**	**1.007**	0.398	0.999	0.911	0.471	1.327	1.867	0.493
1: 800	**0.648**	**0.652**	0.102	0.867	0.900	0.409	0.721	0.782	0.424	**1.369**	**1.417**	0.455
Note: Antibody titers are in bold.												

The data in Table 1 show that the highest titers of anti-brucellosis antibodies in the studied blood serum samples were found when the wells had been sensitized with a recombinant protein at a concentration of 1 μg / ml. This concentration was further taken for making ELISA in order to study the immunoreactivity of the fusion proteins. The data are shown in Table 2.

Table 2 – Studies of blood sera of immunized mice in indirect ELISA

Antibody titers	*Brucella spp* recombinant fusion proteins used to immunize mice								
	Omp25-Omp31 (n = 10)			Omp25-Omp19 (n = 10)			Omp 31 -Omp19 (n = 10)		
	ODi	ODbi	ODi / ODbi	ODi	ODbi	ODi/ ODbi	ODi	ODbi	ODi/ ODbi
200	0.839	0.478	1.75	2.326	0.483	4.82	1.923	0.489	3.93
400	0.655	0.313	**2.09**	1.888	0.350	5.39	1.752	0.376	4.66
800	0.571	0.325	1.76	1.454	0.341	4.26	1.503	0.294	5.11
1600	0.485	0.332	1.46	1.106	0.360	3.07	1.375	0.315	4.37
3200	0.435	0.359	1.21	0.779	0.330	**2.36**	1.135	0.317	3.58
6400	0.528	0.392	1.34	0.533	0.355	1.5	0.883	0.333	**2.65**
12 800	0.516	0.374	1.37	0.330	0.330	1.0	0.630	0.334	1.89
Notes: ODa – average OD for the group of immunized mice; ODK – average OD for the group of mice before immunization; Positive results are shown in bold.									

As it is seen from Table 2, among the studied proteins, Omp31-Omp19 turned out to be the most immunogenic. Omp31-Omp19 caused the formation of specific antibodies in laboratory animals up to titers of 1: 6400. Antibodies against Omp25-Omp19 were detected to a serum dilution of 1: 3200. The immunogenicity of Omp25-Omp31 was comparatively low – 1: 400.

Thus, the recombinant fusion proteins of *Brucella spp.* Omp31-Omp19 and Omp25-Omp19 are characterized by expressed immunoreactive properties and can be used in the development of ELISA tests for serological diagnosis of brucellosis.

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