Evaluate the immunogenicity antibrucellar vaccine vector model of guinea pigs

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Abstract. As a result of extensive research, the highest immunological parameters were obtained in the groups of animals immunized with the mixture of designs NS1-124-L7L12 + NS1-124-Omp 16 (H5N1 and H1N1), not inferior piece of the vaccine. B.Abortus 19, which will be recommended for testing in cattle. In determining the immunogenicity of the vaccine constructs selected the most suitable candidate for further research on cattle, because in this group of relatively low II 4.8 and 60% protection.
[Yespembetov B.A., Syrym N.S., Zinina N.N., Tabynov K.K., Nissanova R.K., Sarmykova M.K., Bezrukova S.Z., Konbaeva G.M. **Evaluate the immunogenicity antibrucellar vaccine vector model of guinea pigs.** *Life Sci J* 2014;11(6s):565-568] (ISSN:1097-8135). http://www.lifesciencesite.com. 118

Keywords: brucellosis, vaccine, vector, immunogenicity, B. abortus, R-form.

Introduction

Despite the improvement of the epizootic situation of brucellosis animals in our country, the problem of improvement of livestock not fully resolved. Identification of infected animals and dysfunctional points not only reduced, but also tends to increase. Found that over the past four years (up 2008 to 2012). The republic registered an average of 952 epizootic outbreaks of brucellosis in small ruminants (MDD) and 135 - seat of brucellosis in cattle (cattle) [1].

Unfortunately, our country today occupies a leading position on the prevalence of the disease among the people - the third after Spain and Kyrgyzstan. In recent years, Kazakhstan has recorded cases of the disease each year 2500-3500 (in neighboring Russia with a population ten times more than in Kazakhstan, only 300-400 cases). [2].

To join the WTO, it was decided to eliminate the vaccination in Kazakhstan, as a result of this decision, the country began mass animal diseases from which people become infected. Today, out of the critical situation of brucellosis in the country is seen only in vaccination. Moreover, the same European Union, which today is a region free of brucellosis in animals, 50 years ago, began his strategy to fight this infection is a mass vaccination of animals [3].

Against brucellosis at various times it was suggested a significant number of dead and live vaccines (B. abortus RB-51 to R-form, B. abortus 75/79-AB, B. abortus 82, B. abortus 19, etc.) [4]. A significant disadvantage of these vaccines is the presence in the blood of immunized animals antibodies detected in serological tests (agglutinogens) abortogennost, short and ineffective protection of immunity against the infection.

In order to address these deficiencies annually to develop new vaccines against brucellosis, such as DNA vaccines, vector vaccines [5,6,7,8]. In view of the members of the Institute RIBSP developed a vaccine against brucellosis in cattle on the basis of a recombinant influenza virus vector.

In this regard, our task was to - to choose the most suitable design series vector vaccine for further testing to the study of cellular and humoral immunity factors.

From the above it follows that the determination of the immunogenicity of vaccines against brucellosis, using advances in modern biology is an important task of Veterinary Biotechnology.

Methods

The immunogenicity of recombinant viral vectors with designs Flu-NS1-124-Omp16 (H5N1, H1N1), Flu-NS1-124-L7/L12 (H5N1, H1N1), and their mixtures were determined in guinea pigs with a live weight of 300-350 gr by their intranasal, conjunctival or subcutaneous administration. Prime vaccination for H5N1 is made designs with intranasal, subcutaneous, conjunctival routes of administration at doses ranging from 5.89 to 6,28 log10 EID50. A booster dose of vaccine constructs for H1N1, depending on the mode of administration, ranged from 6,39 to 7,00 log10 EID50.

Serology (agglutination test, Rose-bengal assay and complement fixation test) was performed using a set of firm "Microgen" Russia.

Hematologic studies were performed by standard methods. Calculation of the absolute number of leukocytes was performed in a Goryaev chamber least 100 large squares, after 10 minutes of incubation 20 microliters. blood at 37 ° C in 0.4 ml of

5% acetic kiloty colored with methylene blue. Leukocyte count was carried out in a smear stained with Romanovsky-Giemsa under magnification MC300X Micros (infinitive), Austria x 1000.

To evaluate the immune status of immunological parameters were used, including certain cytokines. Immunophenotypic analysis of lymphocyte subsets was performed by a flow cytometer FACS Calibur firm «Becton Dickinson».

(Belgium) with the software Cell Quest. The method is based on the interaction of monoclonal antibodies labeled with a fluorescent label, a lymphocyte surface antigens, and subsequent analysis of the samples by flow cytometer. The work was done in the LP "GEM", Almaty.

The immunogenic properties of the test vaccine candidates examined in accordance with generally accepted methods, determining the level of protection against immunization with parallel serological (manifestations of humoral) immunokletochnymi and bacteriological (indication of the pathogen) research. Challenged with virulent made reference strain B. 544 abortus subcutaneously in a dose - 50 MK. / ml. Infection index (AI), immunogenicity was determined by inoculation of Brucella bodies on the 30th day after challenge. The results of bacteriological tests were evaluated by the number of guinea pigs that are not allocated crops (percentage of non infected animals or immunogenicity), the number of crops per infect a pig, and the intensity of colonization (infection) of animals, which is calculated by the following formula:

$$x = \frac{a \times 100}{b \times c}$$

x - The index of infection (II);

a - the number of isolates;

b - number of guinea pigs in the experiment;

c - number of organs and lymph nodes taken for sowing.

Vector constructions *	The method of administration, the number of	Study of serum PEIL PCR n PA	Immunological parameters of blood									Subpopulat. of lymphocyte	
			γ interf Up to 50pg |ml	α-TNF Up to 25 Units	leukocyte 5-12 x 10^3 /1^3	Neyt.μ/l 0-4%	Neutr.s/l 20-40%	monocyte 8-12 %	eosinophil 1-5%	lymphocyte 45-80%	basophil 1-2%	CD-4 + (T Hel) 31-46%	CD-8 - (Tsup c) 23-40%
NS1-124-Omp 16	1 gr.I.N	-	17,1	14.8	10,6	4	44	10	6	40	2	15	13
	2 gr.C	-	14.1	10.4	7,5	5	35	13	9	36	2	6	21
	3rp.S.C	-	13.8	12.8	4,4	-	32	8	2	54	4	17	8
NS1-124-	4 gr.I.N	-	7,12	5,75	4,8	-	40	7	1	51	2	4	21
	5 gr. C.	-	6.51	13.5	5,4	-	41	3	5	47	4	21	15

The results of research

Some Figures nonspecific cellular immune responses to the influenza virus vector construct bearing the antigenic determinants of brucellosis and of the changes in the body of guinea pigs are shown in Table 1.

Table 1. Results of immunological tests of guinea pigs after revaccination

L7L12	6 gr. S.C	-	6.83	6.91	9,2	-	46	10	4	38	2	19	26
mixture NS1-124-L7L12 + NS1-124-Omp 16	7 gr.I.N	-	7.89	7.08	17,7	1	48	1	2	30	-	37	29
	8 gr .C.	-	8.29	5.75	12	1	34	-	-	65	-	20	24
	9 gr S.C	-	4.96	7.46	17.1	2	31	2	8	57	-	38	32
B.ab19	10 gr . S.C	+	6.78	6.71	14,5	4	40	1	5	50	-	34	25
Intact	11 gr .	-	-	4,45	24,0	2	39	-	2	20	-	36	38

Note: I.N - intranasally, C - conjunctival, S.C - subcutaneously.

• - design by serotype H5N1 influenza viruses and the H1N1 (prime, boost).

In analyzing the data from Table 1, given that animals introduced into the body is not brucellosis proteins, and flu-like design on the vector expressing antigens brucellosis, we aimed to: find out the severity of non-specific cell-mediated immunity. At this stage all indicators were compared with the intact group of animals. As can be seen from the results of serological samples of serum from guinea pigs methods Rose-bengal test (RBT) agglutination (Agg) and complement fixation (CFT) in all groups except 10 groups not identified specific agglutinins to Brucella antigen. Then in 10 the group immunized with vaccine volume. B.abortus 19, is already on the 7th day vividly present expressed agglutination RBT and Agg, which is characteristic of the vaccine units. B. abortus. Importantly, the fact that although vector constructions expressing vaccine proteins brucellosis (ribosomal and surface), while in serological tests are nonagglyutinogeneus. This fact is advantageous in that it allows to distinguish between immune responses in serum of patients vaccinated animals from epizootic.

When analyzing leukocyte levels in guinea pigs, it was found a moderate increase in the average number of leukocytes in groups with a mixture of structures NS1-124-L7L12 + NS1-124-Omp 16 (H5N1 and H1N1), which is considered favorable prognostic sign. It is worth noting that increasing the degree of leukocyte concentration depends on the severity of infection and the body's ability to withstand it. The increase of the percentage of lymphocytes (65%) in groups of animals immunized with a mixture of NS1-124-L7L12 + NS1-124-Omp 16 (H5N1 and H1N1), whereas the group immunized with the vaccine strain of B.abortus 19 shows (50%) .

When evaluating the CD-4 + T cells, preferably represented by T helper cells, was a decrease in the overall percentage of all groups other than 7, 8, 9, 10, 11 groups, however, in comparison with a control vaccine volume. B.abortus 19 Group number 7, 8, 9 with a mixture of structures NS1-124-L7L12 and NS1-124-Omp 16 (H5N1 and H1N1), maintained normal blood level of the indicator,

whereas in the groups with 1 - on 6 There has been some decline. A similar pattern is detected by estimating the number of CD8 + lymphocytes with a decrease of this index as a whole, except for groups with a mixture of structures in comparison with the vaccine of the piece. B.abortus 19. In this group of animals immunized with vaccine vector intranasal and subcutaneous administration routes, Omp16 protein expression show the lowest rates -13 and 8%, respectively, at a rate of 23 - 40%, which is due to their redistribution, or development of immunopathological processes immunosuppression.

The content of endogenous interferon in all groups revealed a relatively low titers (2.11 - 17.1 pg / ml) at rate allowable to 50 pg / ml, while the group of intact animals is not detected at all. Interferons play a central role in the differentiation and activation of effector cells of the immune system, so the suppression of interferon plays a pathogenic role in the development of lingering forms of brucellosi

Stab neutrophils are found in all groups except the intact pigs, and the smallest number of them noted in the groups with a mix of designs. The appearance of immature neutrophils in the blood determines the severity of the disease, when the body begins to consume more immature immune cells.

Thus, as a result of immunological tests in guinea pigs, comprising determination of humoral (antibody) and cellular (cytokines leykoformula) units, when compared to intact animals, the group immunized with strain 19 B.abortus generally observed nonspecific immunological tension almost all groups of animals caused by the introduction of virus into the body construction with Brucella inserts.

Further study of the immunogenicity of the vaccine candidate, after the prime - boost immunization with influenza-like structures were determined by generally accepted the challenge with virulent reference strain B. 544 abortus administration subcutaneously in a dose of 50 microbial cells. / ml. On day 30 after challenge all the guinea pigs were sacrificed by CO2 asphyxiation, aseptically opened for taking the lymph nodes, liver, kidney, spleen and bone marrow seeding to further define and Brucella AI immunogenicity. The results are shown in Table 2.

The studies (Table 2) it was found that in comparison with the control group, all samples vaccines II indicator and the percentage of infected animals do not (regardless of the route of administration) to some extent protect guinea pigs against infection pieces. B. abortus 544. Thus the best protection of the experimental groups of guinea pigs was achieved in groups of animals vaccinated conjunctival method monovalent protein structure Omp16 (H5N1 and H1N1), (AI - 6.2 immunogenicity - 60%) and conjunctival method bivalent viral constructs expressing proteins Omp16 + L7/L12 (H5N1 and H1N1), (II 4.8 immunogenicity, 60%), whereas the comparator being the vaccine strain of B. abortus 19 (II 1,7-immunogenicity 50%). The lowest rates of immunogenicity (II 13.7, immunogenicity, 20%) were observed in the group of animals immunized intranasally with monovalent viral constructs Flu-NS1-124-Omp16 (H5N1 + H1N1). Hoping to test the immunogenicity of the vaccine candidate in comparison with the existing vaccine from pc. B. abortus sht.19 received between 0 and 60% of the body's defense against a virulent strain.

Table 2. Performance test the immunogenicity of candidate vaccines

Vector constructions *	method of administration	Number of animals in experience	Infected, heads	Emphasis cultures	Infection index (II)	Immunogenicity %
Flu-NS1-124 -Omp 16	I.N	5	4	4	13.7	20
	C.	5	2	2	6.2	60
	S.C.	5	3	3	9.7	40
Flu-NS1-124-L7L12	I.N	5	3	3	10.6	40
	C.	5	5	5	16.8	0
	S.C.	5	4	4	11.5	20
Flu-NS1-124-Omp 16 + Flu-NS1-124-L7L12	I.N	5	3	3	9.7	40
	C.	5	2	2	4.8	60
	S.C.	5	1	1	13,3	20
B.Abortus 19	S.C.	5	2	2	1.7	50
Control (PBS)	S.C.	5	5	5	19.1	0

Note: I.N - intranasally, K - conjunctival, P.K - subcutaneously.

• - design by serotype H5N1 influenza viruses and the H1N1 (prime, boost).

In the next series of studies was assessed by the degree of protectivity inoculation of a virulent strain of B. abortus 544 from the spleen of animals, the results of which are given in Table 3.

Table 3. The degree of protective vaccines, estimated by the inoculation of Brucella from the spleen of guinea pigs infected with a virulent strain of B. abortus 544

Vector constructions *	method of administration	Log_{10} m.b./ spleen (C3±CO)	Log_{10} protection	Value (*P*)
Flu-NS1-124 -Omp 16 (H_2N_1 + H_1N_1)	I.N.	2.0±0.52	2.54	<0.01
	C.	0.76±0.44	3.78	<0.001
	S.C.	1.26+0.52	3.28	<0.005
Flu-NS1-124-L7L12 (H_3N_1 + H_1N_1)	I.N.	1.22±0.50	3.32	<0.001
	C.	2.4+0.25	2.14	<0.005
	S.C.	1.46±0.39	3.08	<0.001
Flu-NS1-124 -Omp 16 + Flu-NS1-124-L7L12 (H_3N_1 + H_1N_1)	I.N.	1.28+0.52	3.26	<0.005
	C.	0.64±0.40	3.90	<0.001
	S.C.	1.68+0.51	2.86	<0.005
B. *abortus* 19	S.C.	0.42±0.26	4.12	<0.001
Control (PBS)	S.C.	4.54+0.43	0.00	

Note: W - average, CO - standard error, • - design by serotype H5N1 influenza viruses and the H1N1 (prime, boost); m.b - microbial bodies.

The results showed that all samples vaccines, including monovalent virus structure showed the worst results II, regardless of the route of administration, compared with the control group (B. abortus 19) provides more significant protection than marine pigs. It should be noted that in terms of the above viral inoculation design in no way inferior piece of the vaccine. B. abortus 19.

Conclusion

Currently, mainly antibrucellar immunogenicity of vaccines tested using serological

tests, informational content that is unreliable because of the presence of blocking antibodies, the phenomenon of "Prozone", as well as the presence in the body of heterologous microorganisms that cause false positive serological tests for brucellosis. In the formation of antibodies antibrucellar immunity does not belong to the leading and only a supporting role, which is to create antibrucellar no immunity is not involved [10]. We found that immunization of animals mono-and bivalent viral structures makes it possible not only to effectively differentiate vaccinated from infected zhivonyh, but also makes it possible to identify the population of patients with brucellosis vaccinated animals in the herd.

Established that a key role in establishing immunity belongs antibrucellar cellular immune factors, in particular T-cell system including T lymphocytes (T helper cells, T-suppressor) and main - T-killer (cytotoxic T lymphocytes) [9 10,11]. Therefore, to assess the immune status with brucellosis used above immunological tests along classical serological reactions and bacteriological methods.

Results of experiments conducted on 60 guinea pigs immunized candidates - vaccines subsequent contamination piece. B. abortus 544 show that the most pronounced protective effect was observed in experiments with a mixture of vaccine candidates from Flu-NS1-124 - Omp 16 + Flu-NS1-124-L7L12 (H5N1 and H1N1). This is confirmed not only by the number of animals resistant to infection, but also the severity of immunological reactions.

Thus, these data indicate that the active version of the vaccine has a higher immunogenicity compared to other vaccine candidates and can be used in further immunogenicity tests on cattle.

Findings

1. First developed antibrucellar vector vaccines have been evaluated in terms of cell-mediated immunity. Results of studies have shown a protective, that in terms of viral inoculation designs are not inferior piece of the vaccine. B. abortus 19.
2. Proved innagglyutinogennost antibrucellar vector vaccine candidates that can distinguish vaccinated animals from sick.
3. As a result of extensive research, the highest immunological parameters were obtained in the groups of animals immunized with the mixture of designs NS1-124-L7L12 + NS1-124-Omp 16 (H5N1 and H1N1), not inferior piece of the vaccine. B.Abortus 19, which will be recommended for testing in cattle. In determining the immunogenicity of the vaccine constructs selected the most suitable candidate for further research on cattle, because in this group of relatively low II 4.8 and 60% protection.

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4/24/2014