The use of polyazolidineammonium and dimethyl-sulfoxide antigen *Yersinia pseudotuberculosis* to obtain hyperimmune serum

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Abstract. The use of polyazolidinammonium modified with iodine hydrate ions (PAAG) as an adjuvant made it possible to obtain rabbit hyperimmune blood serums for dimethyl-sulfoxide antigen (DA) of a pseudotuberculosis microbe with generic specificity. Antibody titers in ELISA with *Y. pseudotuberculosis* and *Y. enterocolitica* cells amounted to 1:25600-1:12800, and with cells of other genera of intestinal microflora - 1:100-1:400. The optimal immunizing dose for obtaining hyperimmune yersiniosis serum was a dose of 2 mg DA of *Y. pseudotuberculosis* per rabbit. Such a dose made it possible to obtain hyperimmune sera with a high titer of specific antibodies with a small consumption of antigen. The optimal concentration of PAAG solution for hyperimmunization of *Y. pseudotuberculosis* DA rabbits was 1%.

1 Introduction

Intestinal yersiniosis is registered in many countries of the world and occurs in pigs with a large livestock population. In addition to pigs, the circulation of *Yersinia enterocolitica* (*Y. enterocolitica*) is detected in other domesticated animals and birds. However, pigs are the main source of *Y. enterocolitica* for human infection [1-7].

There is less information on the circulation of the pseudotuberculosis microbe in animals than on the circulation of the causative agent of intestinal yersiniosis. Pseudotuberculosis in animals occurs sporadically or in the form of small outbreaks. Infection of people with *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) occurs through an alimentary route mainly through plant products and the role of animals in human infection is not clear [1-4].

Studies by a number of scientists indicate the possibility of simultaneous circulation of both pathogens in the intestines of pigs [3, 4]. At the same time, diagnostic preparations are in demand, allowing simultaneous indication of *Y. enterocolitica* and *Y. pseudotuberculosis*. Such drugs are based on hyperimmune serums with generic specificity.

Hyperimmune sera are obtained by repeated immunization of animal producers with a

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mixture of antigen and adjuvant.

Dimethyl-sulfoxide antigen (DA) can be used to obtain blood serums with generic specificity. This antigen was first studied in *Mycobacterium tuberculosis* [8]. We then isolated and studied DA of *Y. enterocolitica* and *Y. pseudotuberculosis*, as well as antibodies to them [9, 10]. The antibodies obtained from *Y. enterocolitica* allowed us to create two diagnostic test systems based on them [11, 12], the successful tests of which showed the potential for further research in this area.

Recently, synthetic polyelectrolytes have gained popularity as adjuvants. The simplicity of chemical synthesis, solubility in water, and the ability to form conjugates with antigen particles have opened up prospects for their use as adjuvants [13, 14]. One of the representatives of this group of chemical compounds is polyazolidinammonium modified with iodine hydrate ions (PAAG). It has a wide range of antimicrobial properties [15] and is safe for warm-blooded animals [16]. A complex adjuvant consisting of PAAG and microparticles of calcium carbonate was developed for vaccination of animals [17]. The possibilities of using PAAG as an adjuvant for obtaining hyperimmune blood serum were first studied by us when immunizing rabbits with lipopolysaccharide and disintegrated membranes of *Y. pseudotuberculosis* [18, 19]. This experiment showed the promise of using PAAG for hyperimmunization. The aim of our study was to determine the possibility of using PAAG in combination with DA *Y. pseudotuberculosis* to obtain rabbit hyperimmune pseudotuberculous serum.

Experiment design:

1. Multiple immunization of rabbits with different doses of DA in combination with PAAG to determine the optimal immunizing dose of *Y. pseudotuberculosis* DA.
2. Multiple immunization of rabbits with DA using various concentrations of PAAG to determine the concentration of the drug with the highest adjuvant properties.
3. The study of the specificity of the obtained hyperimmune sera in ELISA.
4. Based on the analysis of the results of ELISA and leukocyte counting, it is possible to draw a conclusion about the effectiveness of the use of PAAG in combination with DA *Y. pseudotuberculosis*.

2 Materials and methods

To obtain DA, a microbial culture of *Y. pseudotuberculosis* III O:3 serovariants from the museum collection of pathogenic microorganisms of the Federal Research Institution of Health Protection and Health Research of Russia "Microbe" was used, which has characteristic morphological, cultural, biochemical and serological properties.

The method for obtaining the DA of a pseudotuberculosis microbe consisted in treating the dry acetone microbial mass of bacteria with dimethyl-sulfoxide, followed by taking the liquid, releasing it from dimethyl-sulfoxide and lyophilization [9].

Immunization of male rabbits weighing 2.5 kg of the Chinchilla breed was carried out subcutaneously along the back at 3-4 points in a volume of 1 ml of the mixture. The ratio of adjuvant to antigen solution was 1:1. There were 5 immunizations with an interval of 2 weeks. Blood for the study was taken from the ear vein in a volume of 5 ml a day before the introduction of the antigen, starting with 1 immunization.

The resulting hyperimmune rabbit blood serum was studied by the method of solid-phase indirect ELISA.

The number of leukocytes, lymphocytes and granulocytes in the blood of rabbits was determined on a hematological analyzer.

As antigens for ELISA, in the study of sera, DA *Y. pseudotuberculosis* III at a concentration of 20 µg / ml and formalized cells: *Y. pseudotuberculosis* I O:1 serovariant, *Y. pseudotuberculosis* III O:3 serovariant, *Y. pseudotuberculosis* IV O:4 serovariant,

Y. pseudotuberculosis V O:5 serovariant (*Y. pseudotuberculosis* O:1, O:3, O:4, O:5), *Y. enterocolitica* 66-82 O:3 serovariant, *Y. enterocolitica* 383 O:9 serovariant (*Y. enterocolitica* O:3, O:9), *Enterobacter aerogenes* ATCC-13048, *Escherichia coli* 4295, *Proteus vulgaris* 19, *Salmonella typhimurium* 1626 (obtained from GKPM FGUZ RosNIPCHI "Microbe" Rospotrebnadzor), as well as a single brucellosis antigen production of JSC "Pokrovsky plant of biological products" on the basis of *Brucella abortus* were used. Bacterial suspensions were prepared with a concentration of 1 billion cells / ml.

3 Results

To determine the optimal immunizing dose of DA for *Y. pseudotuberculosis*, rabbits were divided into 6 experimental and 6 control groups, 3 rabbits in each group. Animals from each of the 6 groups were immunized five times with one of the DA doses: 0.2, 1, 2, 4, 8, or 16 mg / animal. Before immunization, the rabbits of the experimental groups were mixed with the antigen 1:1 with 1% solution of PAAG (DA + PAAG), and the rabbits of the control groups were mixed with physiological saline (DA + PS). The obtained blood serum was examined by ELISA in reaction with DA *Y. pseudotuberculosis* (20 µg / ml) (Table 1).

Table 1. The results of immunization of rabbits with different doses of DA *Y. pseudotuberculosis*.

№ immuni- - zation	Antibody titers of the obtained sera after immunization						
	adjuvant (PAAG 1%)	Dose DA in the group, mg / rabbit					
		0.2	1.0	2.0	4.0	8.0	16.0
1	PS (control)	1:400	1:1600	1:1600	1:1600	1:3200	1:3200
	PAAG (test)	1:1600	1:3200	1:3200	1:3200	1:3200	1:3200
2	PS (control)	1:1600	1:6400	1:6400	1:6400	1:12800	1:25600
	PAAG (test)	1:12800	1:25600	1:25600	1:25600	1:25600	1:25600
3	PS (control)	1:3200	1:12800	1:25600	1:25600	1:51200	1:102400
	PAAG (test)	1:25600	1:51200	1:102400	1:102400	1:102400	1:102400
4	PS (control)	1:6400	1:25600	1:25600	1:51200	1:102400	1:204800
	PAAG (test)	1:51200	1:102400	1:204800	1:204800	1:204800	1:204800
5	PS (control)	1:6400	1:25600	1:51200	1:102400	1:204800	1:409600
	PAAG (test)	1:51200	1:204800	1:409600	1:409600	1:409600	1:409600

As can be seen from table 1, in the control groups, the increase in antibody titer is directly proportional to the increase in the dose of DA and the number of immunizations. In the experimental groups, the action of PAAG cancels the dependence of the antibody titer on the dose of DA in the range of 2-16 mg / rabbit, and at doses of 0.2-1 mg / rabbit this dependence is not as pronounced as in the control groups.

The use of PAAG allows in experimental groups to obtain, after 5 immunizations, serums with a higher antibody titer than in the control: when immunized with doses of 0.2-2 mg / rabbit DA, the values of the experimental group titers are 8 times higher than the control titer, with a dose of 4 mg / rabbit DA – 4 times, with a dose of 8 mg / rabbit – 2 times. However, with a significant dose of DA (16 mg / rabbit), the effect of PAAG on the antibody titer is absent.

The highest antibody activity is serum obtained from rabbits immunized with DA in doses of 2-16 mg / rabbit using PAAG. The titers of these sera were 1:409600.

In rabbits immunized with various doses of DA *Y. pseudotuberculosis*, after 5 immunizations, blood was additionally examined to calculate the total number of leukocytes (WBC), as well as their two types: lymphocytes (L) and granulocytes (G) (Table 2).

Table 2. The results of determining the number of leukocytes in the blood of rabbits immunized with various doses of DA *Y. pseudotuberculosis*.

Doses DA mg / rabbit	The number of leukocytes in the blood of a rabbit (10 ⁹ cells / liter)					
	Presence of adjuvant (PAAG 1%)					
	PAAG (test)			PS (control)		
	WBC	L	G	WBC	L	G
2.0	7.9	3.2	3.5	5.0	2.8	2.0
4.0	11.5	4.8	5.8	–	–	–
8.0	15.6	5.7	8.5	–	–	–
16.0	16.2	6.3	8.9	15.8	4.9	9.0

As can be seen from table 2, an increase in the number of leukocytes is affected by an increase in the immunizing dose of DA *Y. pseudotuberculosis*, as well as the use of PAAG. PAAG has a more pronounced stimulating effect on lymphocytes than on granulocytes.

The optimal concentration of PAAG was determined by immunization of 3 groups of rabbits, which were injected with 2 mg of DA *Y. pseudotuberculosis* in a mixture with various concentrations of PAAG (0.2%, 1%, 5%) in a 1:1 ratio. Rabbits were immunized as described above. The blood serum obtained after 5 immunizations was tested by ELISA in response to DA *Y. pseudotuberculosis* (20 µg / ml).

Sera obtained from rabbits immunized using a 0.2% PAAG solution had a titer of 1:51200, a 1% PAAG solution – 1:409600, and a 5% PAAG solution – 1:102400. Thus, the optimal concentration for immunization is 1% PAAG solution concentration.

The specificity of the sera obtained after 5 immunizations was studied in ELISA with formalized bacterial cells. The results are shown in table 3.

Table 3. The results of the determination of the specificity of the blood serum of rabbits immunized with DA *Y. pseudotuberculosis* and PAAG.

Bacterial cells	Serum antibody titers with bacterial cells diluted 10 ⁹ cells / ml
<i>Y. pseudotuberculosis</i> O:1	1:25600
<i>Y. pseudotuberculosis</i> O:3	1:25600
<i>Y. pseudotuberculosis</i> O:4	1:12800
<i>Y. pseudotuberculosis</i> O:5	1:12800
<i>Y. enterocolitica</i> O:3	1:25600
<i>Y. enterocolitica</i> O:9	1:12800
<i>Escherichia coli</i>	1:400
<i>Salmonella typhimurium</i>	1:100
<i>Proteus vulgaris</i>	1:200
<i>Enterobacter aerogenes</i>	1:200
<i>Brucella abortus</i>	1:200

In the study of the specificity of rabbit blood serum obtained using PAAG and DA *Y. pseudotuberculosis*, showed high titers of antibodies with cells of *Y. pseudotuberculosis* and *Y. enterocolitica*, as well as low titers with cells of other genera of intestinal microflora and brucella, which indicates the genus specificity of the data serums.

4 Discussion

For hyperimmunization of rabbits with DA *Y. pseudotuberculosis* and PAAG, 2 mg DA / rabbit should be used as the main immunizing dose, as such a dose allows for a small consumption of antigen to obtain hyperimmune serum with a high titer of specific antibodies. However, it should be noted that when using high doses of DA (16 mg / rabbit), the use of an adjuvant is not required, because the composition of DA *Y. pseudotuberculosis* includes proteins that aggregate in concentrated solutions, increasing their antigenicity.

The mechanism of action of PAAG on antibody genesis is apparently associated mainly with an increase in the rate of antigen presentation, lymphocyte differentiation, and to a lesser extent due to an increase in the number of lymphoid cells. This assumption is indicated by high titers of specific antibodies with a relatively low number of lymphocytes in the blood.

The optimal concentration of PAAG solution for immunization is 1%. Exceeding this concentration by 5 times (5% PAAG solution) is accompanied by the appearance of a smell of iodine. An excess of which, apparently, negatively affects the properties of DA and the local reaction of the body, which leads to a decrease in the titer of specific antibodies.

The results of ELISA with various bacteria indicate the yersiniosis specificity of the

obtained serums. Low titers of antibodies to bacteria of other genera do not have a significant effect on the specificity of the sera.

5 Conclusions

Based on the work done, we can draw the following conclusions:

1. The use of PAAG in combination with DA *Y. pseudotuberculosis* allows to obtain hyperimmune rabbit blood serum with a high titer of specific antibodies.
2. The resulting hyperimmune serum has yersiniosis specificity.
3. The optimal immunizing dose to obtain hyperimmune yersiniosis serum using PAAG as adjuvant is a dose of 2 mg DA *Y. pseudotuberculosis* per rabbit.
4. The optimal concentration of PAAG solution during hyperimmunization of DA *Y. pseudotuberculosis* rabbits is 1%.

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