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Study of Properties of Polyvalent Serum of Intestinal Yersinosis Obtained from Experimental Animals Using Different Immunization Schemes

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Abstract The article describes the results of obtaining polyvalent serum in experimental animals by hyperimmunization, as well as the dynamics of total protein, albumin, globulins, IgA, IgM, IgG indicators, serum activity using extended agglutination reaction in slides and tubes at the stages of hyperimmunization. An increase in the level of globulins was observed as an immune response in experimental animals on days 7-14-21-28 of immunization. In blood sera obtained from experimental animals co-injected with inactivated corpuscular and soluble antigens of serovar strains of Yersinia enterocolitica, high levels of total protein, albumin, globulin and IgG were observed on the 28th day of the experiment.

Keywords Intestinal yersiniosis, *Yersinia enterocolitica*, Hyperimmunization, Polyvalent serum, Total protein, Albumin, Globulin, IgA, IgM, IgG, Agglutination, Antigen

1. Introduction

Currently, bacterial infections are a global problem. An example among such bacteria that cause dangerous infections is *Yersinia*. *Yersinia* is a bacterium from the family *Enterobacteriaceae*, gram-negative bacilli, facultative anaerobes. It includes 18 species, the most common of which are: *Yersinia pseudotuberculosis, Yersinia enterocolitica* and *Yersinia pestis* (causative agent of plague) [1,2,6,10]. Enteropathogenic *Yersinia* include the causative agents of pseudotuberculosis and intestinal yersiniosis. The causative agent of intestinal yersiniosis is Yersinia enterocolitica. The main reservoir of the pathogen is rodents [3,4].

The bacteriological method used in the diagnosis of intestinal yersiniosis requires high labor intensity, long periods of pathogen isolation, low efficiency, gives a large number of false isolations of yersiniosis cultures, which is associated with significant contamination of pathological material with intestinal microflora and imperfection of isolation methods. Serologic diagnostic methods significantly increase the efficiency of *Yersinia* isolation [5,7].

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Standard sera are important for the efficiency of serologic testing. Standard sera are obtained by hyperimmunization.

Hyperimmunization is a method of parenteral administration of increasing doses of the corresponding antigens to animals in order to obtain the highest immunological response of the organism and, consequently, the maximum increase of specific antibodies in the blood of animals, which should provide therapeutic, prophylactic and diagnostic effect of preparations [8,11].

In order to improve measures against intestinal yersiniosis, it is necessary, first of all, to improve methods of diagnostics of this infection. Agglutinating sera should be obtained to detect the causative agents of the main serovars (*Yersinia enterocolitica O5, Yersinia enterocolitica O9*) obtained from patients and the external environment. This, in turn, creates the basis for early and timely diagnosis of intestinal yersiniosis and appropriate preventive measures.

The purpose of the study is to investigate the properties of polyvalent sera of intestinal yersiniosis obtained from experimental animals under different immunization schemes.

For hyperimmunization 12 rabbits weighing from 2.3 to 3.3 kg at the age of 4 to 6 months were used. The experiments were conducted in accordance with the methodological manual [9] approved by the Ministry of Health of the Republic

of Uzbekistan in 2016 “Methods and rules for working with laboratory animals in experimental microbiological and immunological studies”.

2. Bacteriologic Method

Serovars *O3 Yersinia enterocolitica 005011/659* and *O9 Yersinia enterocolitica 005008/656* were used for serum production. Experimental animals were injected with a suspension of cultures of these strains prepared at various concentrations according to the McFarland standard and cultured on neutral agar. The experimental animals were quarantined for 21 days.

The rabbits were divided into 4 groups. 3 rabbits were taken into each group:

- in group I at the 1st immunization rabbits were inoculated with *Yersinia enterocolitica 005011/659* strain of serovar O3 corpuscular microbial cells with a concentration of 4 billion cl/mL, at the 2nd 8 billion cl/mL, at the 3rd 16 billion cl/mL, at the 4th 20 billion cl/mL and at the 5th 25 billion cl/mL,
- in group II at the 1st immunization was inoculated with *Yersinia enterocolitica 005011/659* strain of serovar O3 with a concentration of 4 billion cl/mL of soluble and corpuscular antigen in a ratio of 1:1, at the 2nd 4 billion cl/mL of soluble antigen, at the 3rd 16 billion cl/mL of soluble and corpuscular antigen. cl/mL of soluble and corpuscular antigen in a 1:1 ratio, at the 4th 20 billion cl/mL of soluble and corpuscular antigen in a 1:1 ratio, and at the 5th 25 billion cl/mL of soluble antigen,
- in group III at the 1st immunization was inoculated with *Yersinia enterocolitica 005008/656* strain O:9 of corpuscular microbial cells with a concentration of 4 billion cl/mL, at the 2nd 8 billion cl/mL, at the 3rd 16 billion cl/mL, at the 4th 20 billion cl/mL and at the 5th 25 billion cl/mL,
- in group IV at the 1st immunization was inoculated with *Yersinia enterocolitica 005008/656* strain of serovar O:9 with a concentration of 4 billion cl/mL of soluble and corpuscular antigen in a ratio of 1:1, at the 2nd 4 billion cl/mL of soluble antigen, at the 3rd 16 billion cl/mL of soluble and corpuscular antigen. cl/mL of soluble and corpuscular antigen in a 1:1 ratio, at the 4th 20 billion cl/mL of soluble and corpuscular antigen in a 1:1 ratio, and at the 5th 25 billion cl/mL of soluble antigen..

Each of 12 rabbits in each of the 4 groups was injected with 1.6 ml of inactivated *Yersinia enterocolitica* culture or soluble antigen at 8 points: the first 4 points were injected subcutaneously along the spine on both sides (1-4), the next 4 points were injected intramuscularly into the muscles of both front and hind legs (5-8). The volume in each point was 0.2 ml.

Serological method: Conducted on the basis of the order of the Ministry of Health of the Republic of Uzbekistan № 170 from April 19, 2004 “On improvement of measures to combat yersiniosis”.

Immunologic studies. A reagent kit (Vector Best, Russian Federation) was used for immune-enzymatic determination of total immunoglobulins A, M and G, as well as immunoglobulins of classes M and G to intestinal *Yersinia* pathogens. The results were evaluated according to the manufacturer's instructions.

The amount of total protein, albumin and globulin was analyzed by enzymatic-colorimetric method on the biochemical analyzer “Mindray” VA-88A - medical equipment of the Chinese company. The results were evaluated according to the manufacturer's instructions.

Statistical method. The digital material was processed by the method of variation statistics using the program “Excel-Office” 2013 with the use of Student's t-criterion. The mean square error (m) was calculated, as well as the reliability of differences between the values in the compared groups. Differences were considered reliable at $p<0.05$. Nominal data were described with absolute values and percentages. Nominal data were compared using Pearson's $\chi 2$ test, Fisher's exact test. Differences were considered reliable at $p<0.05$.

3. Results and Discussion

Changes in the amount of albumin and globulins in the composition of total protein and protein fractions are considered a response of the organism to hyperimmunization. Globulins are involved in the transport of lipids, hormones, vitamins and metal ions in the body, and their importance in the immune system has been proven. An increase in the level of globulins as an immune response was observed in the body of experimental animals on days 7, 14, 21 and 28 of immunization (Table 1). The increasing trend of total protein content on 7, 14, 21 and 28 days of immunization also indicates the activation of defense mechanism and albumin content increased from 34.03±1.126 g/L to 54.30±0.794 g/L due to the increase in total protein content raised to 70.33±2.345 g/L.

IgM classically affects complement activation. Antibodies of this class are produced when the body is exposed to an infectious agent. The fact that its amount during immunization did not exceed the standard level, indicates that the disease did not develop in the body of the rabbit.

The amount of immunoglobulins A and M in serum increased on days 7 and 14 after immunization and decreased on days 21-28-35. The amount of immunoglobulin G increased on days 7, 14, 21 and 28 after immunization and began to decrease after 35 days. This immunoglobulin provides the body's memory against the infectious agent and provides a secondary humoral response to infection.

The half-life of IgG is 23-35 days. Therefore, after the 5th immunization, i.e. from the 35th day, its amount began to decrease.

No significant changes to the pathogen of intestinal yersiniosis were detected on IgM (p=0.1-0.29) and IgG (p=0.08-0.56).

According to the analysis of blood serum of experimental animals of the 2nd group, the increase of total protein from 91,63±3,631 g/l to 121,9±19,32 g/l on the 7-14-21-28th days of immunization indicates a sharp protective reaction of the organism of experimental animals (Table 2). On the 35th day of the experiment the content of total protein decreased to 60.87±4.468 g/l, which indicates the process of adaptation. The amount of albumin also increased from 33,4±1,15 g/l to 62,13±9,421 g/l on the 7-14-21-28th days of immunization due to the increase of total protein and on the 35th day was 31,2±2,159 g/l.

On the 7-14-21-28th days of immunization, the amount of globulin increased to 58.23±4.269-59.1±9.34 g/L, and on the contrary, this index decreased to 29.67±2.318 g/L on the 35th day.

The amount of immunoglobulin A in serum increased from 0.17±0.009 mg/ml to 0.29±0.075 mg/ml on days 7-14 of immunization and decreased to 0.11±0.006 mg/ml on day 21, to 0.58±0.054 mg/ml on day 28 and to 0.25±0.013 mg/ml on day 35. The above-mentioned changes in the amount of this immunoglobulin in the blood of experimental animals were within the normal range.

The amount of IgM decreased to 0.40±0.006 mg/ml on days 7-14-21 after immunization, to 0.21±0.013 mg/ml on day 28 and to 0.18±0.003 mg/ml on day 35. Considering that IgM is gradually converted to IgG, the amount of IgG increased from 1.15±0.165 to 8.28±0.549 mg/ml on days 7-14-21-28 and to 1.73±0.024 mg/ml on day 35 after immunization and started to decrease again to 0.024. This is an indicator of standard level, which showed the adequacy of immune response in experimental animals.

Significant changes of IgM (p=0.19-1) and IgG (p=0.18 -0.54) indices of the pathogen of intestinal yersiniosis were not revealed.

On 7-14-21-28th days of immunization, the amount of total protein in experimental animals of group 3 increased from 91.07±3.755 g/L to 113.3±15.112 g/L, and the amount of albumin also increased from 33.97±1.92 g/L to 59.13± 7.279 g/L. (Table 3). On the 35th day of immunization, these values were 62.43±4.476 g/L and 32.4±2.219 g/L respectively. Similarly, the amount of globulins increased on the 7th, 14th, 21st and 28th day of immunization and decreased on the 35th day.

It was observed that the amount of immunoglobulin A in serum decreased to 0.12±0.015 and 0.26±0.044 mg/mL respectively on the 7th-14th day, to 0.08±0.003 mg/mL on the 21st day, to 0.30±0.012 mg/mL on the 28th day, and to 0.24±0.13 mg/mL on the 35th day.

The amount of immunoglobulin M was found to decrease from 0.41±0.007 mg/ml to 0.17±0.003 mg/ml on days 7-14-21 after immunization and remained stable at 0.2± 0.003-0.2±0.012 mg/ml on days 28-35.

The increase in IgG from 1.15±0.129 mg/ml to 6.37±0.46 mg/ml on days 7-14-21-28 after immunization indicated a normal immune response in experimental animals.

No significant changes of IgM and IgG indices to the pathogen of intestinal yersiniosis were observed (p=0.38-1; p=0.09-0.9, respectively).

The amount of total protein in blood serum of experimental animals of group 4 decreased from 104.37±6.702 g/l to 96.37±2.369 g/l on the 7th and 14th days of immunization, and from 123.03±5.387 g/l to 64.87±7.496 g/l on the 21st-35th days of immunization. There was a tendency of decrease in total protein indices (Table 4).

Globulin content tended to decrease from 71.13±9.136 g/l to 46.87±0.406 g/l on the 7-14th day of immunization and from 60.57±2.667 g/l to 30.83±5.219 g/l on the 21-35th day. This indicated adaptation processes in the organism of experimental animals.

Albumin content increased from 33,23±3,206 g/l to 62,47±2,72 g/l after 1-3 immunizations, and after 4-5 immunizations it decreased from 58,17±6,288 g/l to 34,03±2,285 g/l. A decreasing trend was observed.

Immunoglobulin A in blood serum was 0.13±0.012 mg/ml on the 7th day after immunization, 0.26±0.019 mg/ml on the 14th day, and on the 21st-28th-35th days of immunization the changes in the amount of IgA in the blood serum of experimental animals were 0.09±0.003 - 0.33±0.007 and 0.26±0.003 mg/ml, respectively. It was proved that the protective reaction of the organism is stronger for 20 bln. amount of soluble and corpuscular antigen.

The amount of immunoglobulin M was stable at 0.40± 0.006-0.37±0.021 mg/ml 7-14 days after immunization, and there was a decreasing trend from 21-28-35 days to 0.17± 0.003-0.19±0.009 mg/ml. The amount of immunoglobulin G increased to 1.08±0.136 - 6.91±0.774 mg/ml on 7-14-21-28 days after immunization and decreased sharply to 1.69± 0.086 mg/ml after the 5th immunization. This situation showed that a stable secondary immune response was formed in the organism of experimental animals.

No significant changes in IgM (p=0.47-1) and IgG to the causative agent of intestinal yersiniosis (p=0.37-1) were observed.

Blood sera obtained from experimental animals (rabbits) of the 4th group on the 7th and 14th days of immunization showed weakly positive results when tested by agglutination reaction on a slide (Table 5).

Blood serum obtained from experimental animals of group 3 was examined by the above reaction and on the 14th day a weakly positive result was observed.

On the 21st day of immunization the result of the agglutination reaction was weakly positive in the blood sera of animals of the 1st and 2nd groups, and positive results were observed when the blood sera of animals of the 3rd and 4th groups were examined by this reaction.

Table 1. Results of immunization of experimental animals of the first group

Indicators	Before immunization	After 1st immunization		After 2nd immunization		After 3rd immunization		After 4th immunization		After 5th immunization	
	M±m	M±m	p	M±m	p	M±m	p	M±m	p	M±m	p
Total protein, g/l	68,37±2,554	97,90±0,624	p=0.001	89,57±0,498	p=0.003	104,80±1,419	p=0.001	106,6±7,123	p=0.01	59,2±3,782	p=0.13*
Albumin, g/l	34,03±1,126	28,77±1,017	p=0.04	45,20±0,361	p=0.002	54,30±0,794	p=0.0006	53,8±3,78	p=0.01	30,6±1,935	p=0.22*
Globulin, g/l	34,33±1,538	69,13±1,102	p=0.0003	44,43±0,348	p=0.007	50,50±0,814	p=0.002	52,8±3,355	p=0.01	28,6±1,85	p=0.09*
A/G, g/l	0,99±0,025	0,41±0,021	p=0.0003	1,01±0,012	p=0.52*	1,07±0,017	p=0.07*	1,01±0,012	p=0.52*	1,06±0,007	p=0.07*
IgA, mg/ml	0,28±0,027	0,15±0,012	p=0.02	0,23±0,037	p=0.35*	0,11±0,007	p=0.008	0,31±0,023	p=0.45*	0,26±0,006	p=0.52*
IgM, mg/ml	1,14±0,090	0,42±0,006	p=0.004	0,34±0,009	p=0.003	0,18±0,012	p=0.001	0,24±0,012	p=0.002	0,18±0,003	p=0.001
IgG, mg/ml	1,07±0,260	0,69±0,086	p=0.25*	0,97±0,212	p=0.78*	1,64±0,028	p=0.11*	5,78±0,236	p=0.001	1,68±0,027	p=0.10*
IgM to the causative agent of intestinal yersiniosis, OD	0,08±0,007	0,10±0,005	p=0.10*	0,10±0,014	p=0.29*	0,11±0,005	p=0.03	0,12±0,021	p=0.16*	0,12±0,003	p=0.01
IgG to the causative agent of intestinal yersiniosis, OD	0,10±0,007	0,12±0,004	p=0.08*	0,12±0,010	p=0.19*	0,13±0,003	p=0.02	0,11±0,014	p=0.56*	0,11±0,004	p=0.30*

Note: p<0.05, reliability of differences with indicators before immunization and after immunization, OD– optical density.

Table 2. Results of immunization of experimental animals of the second group

Indicators	Before immunization	After 1st immunization		After 2nd immunization		After 3rd immunization		After 4th immunization		After 5th immunization	
	M±m	M±m	p	M±m	p	M±m	p	M±m	p	M±m	p
Total protein, g/l	77,87±2,888	91,63±3,631	p=0.05	87,17±14,11	p=0.56*	114,47±10,52	p=0.04	121,9±19,32	p=0.1*	60,87±4,468	p=0.04
Albumin, g/l	38,57±1,445	33,4±1,15	p=0.06	44,97±7,872	p=0.48*	58,03±5,04	p=0.03	62,13±9,421	p=0.08*	31,2±2,159	p=0.05
Globulin, g/l	39,3±1,443	58,23±4,269	p=0.02	42,2±6,278	p=0.68*	56,43±5,538	p=0.05	59,1±9,34	p=0.12*	29,67±2,318	p=0.03
A/G, g/l	0,98±0,003	0,58±0,061	p=0.007	1,05±0,035	p=0.14*	1,03±0,022	p=0.1*	1,05±0,018	p=0.03	1,05±0,015	p=0.01
IgA, mg/ml	0,26±0,033	0,17±0,009	p=0.07*	0,29±0,075	p=0.73*	0,11±0,006	p=0.02	0,31±0,038	p=0.39*	0,25±0,013	p=0.79*
IgM, mg/ml	0,91±0,046	0,40±0,006	p=0.001	0,34±0,042	p=0.002	0,16±0,012	p=0.0005	0,21±0,013	p=0.001	0,18±0,003	p=0.0005
IgG, mg/ml	1,48±0,124	1,15±0,165	p=0.20*	0,88±0,017	p=0.01	1,56±0,074	p=0.61*	8,28±0,549	p=0.001	1,73±0,024	p=0.14*
IgM to the causative agent of intestinal yersiniosis, OD	0,09±0,002	0,09±0,014	p=1.0*	0,09±0,007	p=1.0*	0,1±0,015	p=0.55*	0,11±0,012	p=0.19*	0,12±0,021	p=0.25*
IgG to the causative agent of intestinal yersiniosis, OD	0,09±0,007	0,10±0,006	p=0.35*	0,107±0,012	p=0.30*	0,12±0,016	p=0.18*	0,10±0,008	p=0.41*	0,10±0,013	p=0.54*

Note: p<0.05, reliability of differences with indicators before immunization and after immunization, OD– optical density

Table 3. Results of immunization of experimental animals of the third group

Indicators	Before immunization	After 1st immunization		After 2nd immunization		After 3rd immunization		After 4th immunization		After 5th immunization	
	M±m	M±m	p	M±m	p	M±m	p	M±m	p	M±m	p
Total protein, g/l	71,57±4,114	91,07±3,755	p=0.03	100,47±2,033	p=0.008	106,37±5,393	p=0.01	113,3±15,112	p=0.07*	62,43±4,476	p=0.22*
Albumin, g/l	35,5±2,007	33,97±1,92	p=0.62*	51,37±1,035	p=0.005	53,63±2,521	p=0.01	59,13±7,279	p=0.05	32,4±2,219	p=0.37*
Globulin, g/l	36,07±2,114	57,1±1,877	p=0.005	49,1±1,002	p=0.01	52,73±2,89	p=0.01	56,2±7,535	p=0.08*	30,03±2,315	p=0.14*
A/G, g/l	0,98±0,006	0,59±0,015	p=0.0001	1,04±0,003	p=0.002	1,01±0,02	p=0.24*	1,01±0,009	p=0.06*	1,08±0,029	p=0.04
IgA, mg/ml	0,14±0,015	0,12±0,015	p=0.41*	0,26±0,044	p=0.08*	0,08±0,003	p=0.02	0,30±0,012	p=0.003	0,24±0,13	p=0.50*
IgM, mg/ml	1,01±0,046	0,41±0,007	p=0.001	0,33±0,006	p=0.001	0,17±0,003	p=0.0003	0,2±0,003	p=0.0004	0,2±0,012	p=0.0004
IgG, mg/ml	1,35±0,161	1,15±0,129	p=0.40*	1,13±0,197	p=0.45*	1,57±0,037	p=0.27*	6,37±0,46	p=0.001	1,66±0,031	p=0.15*
IgM to the causative agent of intestinal yersiniosis, OD	0,10±0,010	0,09±0,008	p=0.49*	0,09±0,012	p=0.56*	0,10±0,012	p=1.0*	0,11±0,013	p=0.58*	0,12±0,017	p=0.38*
IgG to the causative agent of intestinal yersiniosis, OD	0,08±0,006	0,10±0,007	p=0.11*	0,11±0,011	p=0.09*	0,11±0,017	p=0.19*	0,09±0,008	p=0.90*	0,11±0,013	p=0.12*

Note: p<0.05, reliability of differences with indicators before immunization and after immunization, OD– optical density

Table 4. Results of immunization of experimental animals of the fourth group

Indicators	Before immunization	After 1st immunization		After 2nd immunization		After 3rd immunization		After 4th immunization		After 5th immunization	
	M±m	M±m	p	M±m	p	M±m	p	M±m	p	M±m	p
Total protein, g/l	74,1±4,823	104,37±6,702	p=0.03	96,37±2,369	p=0.02	123,03±5,387	p=0.01	114,97±12,486	p=0.05	64,87±7,496	p=0.37*
Albumin, g/l	37,07±2,293	33,23±3,206	p=0.40*	49,5±2,291	p=0.10*	62,47±2,72	p=0.005	58,17±6,288	p=0.05	34,03±2,285	p=0.63*
Globulin, g/l	37,03±2,531	71,13±9,136	p=0.03	46,87±0,406	p=0.03	60,57±2,667	p=0.01	50,07±12,724	p=0.38*	30,83±5,219	p=0.36*
A/G, g/l	1,00±0,006	0,49±0,092	p=0.01	1,04±0,037	p=0.36*	1,03±0,003	p=0.02	1,29±0,273	p=0.36*	1,15±0,145	p=0.37*
IgA, mg/ml	0,15±0,015	0,13±0,012	p=0.37*	0,26±0,019	p=0.01	0,09±0,003	p=0.02	0,33±0,007	p=0.001	0,26±0,003	p=0.005
IgM, mg/ml	0,40±0,038	0,40±0,006	p=1.0*	0,37±0,021	p=0.53*	0,17±0,003	p=0.01	0,19±0,006	p=0.01	0,19±0,009	p=0.01
IgG, mg/ml	1,25±0,128	1,08±0,136	p=0.42*	0,93±0,234	p=0.31*	1,78±0,06	p=0.03	6,91±0,774	p=0.005	1,69±0,086	p=0.05
IgM to the causative agent of intestinal yersiniosis, OD	0,10±0,009	0,10±0,014	p=1.0*	0,11±0,01	p=0.51*	0,10±0,014	p=1.0*	0,12±0,023	p=0.47*	0,12±0,007	p=0.17
IgG to the causative agent of intestinal yersiniosis, OD	0,11±0,007	0,10±0,016	p=0.60*	0,13±0,018	p=0.37*	0,11±0,008	p=1.0*	0,11±0,017	p=1.0*	0,11±0,008	p=1.0*

Note: p<0.05, reliability of differences with indicators before immunization and after immunization, OD– optical density

Table 5. Dynamics of antibody titer changes in experimental animals immunized with *Yersinia enterocolitica* strains

Groups of experimental animals	Before immunization		Types and concentration of antigens (number of immunizations)	Динамика антител									
				7th day		14th day		21st day		28th day		35th day	
	A	B		A	B	A	B	A	B	A	B	A	B
1-group	0	0	*Y. enterocolitica 005011/659* serovar O3 (corpuscle) 4,8,16,20,25 billion (1,2,3,4,5 immunization)	negative	negative	o negative	negative	weakly positive	1:133	weakly positive	1:266	Strongly positive	1:800
2-group	0	0	at the 1st immunization inoculated with *Yersinia enterocolitica 005011/659* strain of serovar O3 with a concentration of 4 billion cl/mL of soluble and corpuscular antigen in the ratio 1:1, at the 2nd 4 billion cl/mL of soluble antigen, at the 3rd 16 billion cl/mL of soluble and corpuscular antigen in the ratio 1:1, at the 4th 20 billion cl/mL of soluble and corpuscular antigen in the ratio 1:1. cl/mL of soluble and corpuscular antigen in a 1:1 ratio, at the 4th 20 billion cl/mL of soluble and corpuscular antigen in a 1:1 ratio, and at the 5th 25 billion cl/mL of soluble antigen.	negative	negative	negative	negative	weakly positive	1:50	positive	1:133	Strongly positive	1:400
3-group	0	0	in group III at the 1st immunization inoculated with strain *Yersinia enterocolitica 005008/656* serovar O9 corpuscular microbial cells concentration of 4 billion cl/mL, at the 2nd 8 billion cl/mL, at the 3rd 16 billion cl/mL, at the 4th 20 billion cl/mL and at the 5th 25 billion cl/mL.	negative	negative	weakly positive	1:50	positive	1:400	positive	1:800	Strongly positive	1:11 733
4-group	0	0	in group IV at the 1st immunization inoculated with *Yersinia enterocolitica 005008/656* strain of serovar O9 with a concentration of 4 billion cl/mL of soluble and corpuscular antigen at a ratio of 1:1, at the 2nd 4 billion cl/mL of soluble antigen, at the 3rd 16 billion cl/mL of soluble and corpuscular antigen at a ratio of 1:1, at the 4th 20 billion cl/mL of soluble and corpuscular antigen at a ratio of 1:1. cl/mL of soluble and corpuscular antigen in a 1:1 ratio, at the 4th 20 billion cl/mL of soluble and corpuscular antigen in a 1:1 ratio, and at the 5th 25 billion cl/mL of soluble antigen..	weakly positive	negative	weakly positive	negative	positive	1:300	positive	1:533	Strongly positive	1:4266

Note: A- Agglutination reaction on a slide; B- Extended agglutination reaction in test tubes. The arithmetic mean titer of serologic reactions is given

On the 28th day of immunization examined blood sera of experimental animals of the 2nd, 3rd and 4th groups with the help of agglutination reaction on the slide registered positive results, and blood sera of animals of the 1st group gave sharply positive results.

On the 35th day of immunization, i.e. on the fifth immunization with antigens, all indices gave sharply positive results.

When the blood sera obtained from experimental animals at the stages of immunization were examined by extended in vitro agglutination reaction, the result was observed in the blood sera of group 3 animals in titer 1:50 on the 14th day of immunization.

From the 21st day of immunization, i.e. from the third inoculation with corpuscular and soluble antigens of Yersinia enterocolitica, an agglutination reaction was detected, starting with a mean titer of 1:50 and diluting to a titer of 1:400.

Mean titers ranging from 1:133 (group 2) to 1:11733 (group 3) were observed in the extended agglutination reaction of sera obtained on day 28 (4th immunization and day 35 (5th immunization) of immunization.

4. Conclusions

1. Experimental animals showed an increase in globulins as an immune response on days 7-, 14-, 21- and 28 of immunization.
2. On the 28th day of the experiment, high levels of total protein, albumin, globulin, and IgG were detected in the sera of group 2 animals that were co-injected with corpuscular and soluble antigens of *Yersinia enterocolitica* serovar O3 strains.
3. In serum, immunoglobulin A increased on the 14th-28th day, decreased on the 21st-35th day, immunoglobulin M decreased on the 14th day, decreased on the 21st day, and a stable trend was observed after 28-35 days.
4. No significant changes of IgM and IgG indices in relation to the pathogen of intestinal yersiniosis were revealed.
5. On the 35th day of immunization, i.e. on the fifth immunization with antigens, all indicators gave sharply positive results.
6. After the third immunization, i.e. from the 21st day of immunization, a dynamic increase in antibody titer was observed in all groups when blood sera obtained from experimental animals were examined by in vitro agglutination reaction.
7. Considering that the values of total protein, albumin, globulin and IgG in experimental rabbits are highest on the 28th day of immunization, sera with high titers can be obtained from the animals after the 4th week.
8. To obtain a full-fledged active hyperimmune serum it is necessary to use inactivated corpuscular and soluble antigens together.

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