

DETERMINATION OF THE OPTIMAL IMMUNIZING DOSE OF CONCENTRATED MULTIVALENT INACTIVATED VACCINE AGAINST LEPTOSPIROSIS IN CATTLE

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Scientists of laboratory of Leptospirosis of the Institute of Veterinary Medicine has developed and manufactured one series of experimental multivalent vaccine for cattle, which consists of five serogroups of Leptospira (Icterohaemorrhagiae, Tarassovi, Hebdomadis, Sejroe, Grippytyphosa), which are currently the most widespread on the territory of Ukraine. The article presents the data to determine the optimal immunizing doses of the experimental series of inactivated polyvalent vaccine against leptospirosis in cattle. Antibody response was determined by microscopic agglutination test in dynamics 14, 21, 60, 90 and 180 days after vaccination.

Keywords: *vaccine, leptospira, leptospirosis, cattle, serogroups, strain, antibody, microagglutination test.*

One of the most main prevention methods against Leptospirosis is vaccination. It is prophylaxis of chronic and acute form of disease and carrier state of leptospirosis [1, 2].

Vaccine of leptospirosis is highly effective only if it is composed with serogroups leptospira, which are the causative agents of disease in the definite area. In their absence leptospirosis will occur despite the ongoing vaccination [3–5].

On the territory of Ukraine Leptospirosis in cattle causes by the next serogroups of Leptospira: *Sejroe, Hebdomadis, Icterohaemorrhagiae, Tarassovi* та *Grippytyphosa* [6, 7].

Nowadays for the prophylaxis of leptospirosis in cattle on the territory of Ukraine it is used the polyvalent vaccine VDNKI against leptospirosis of animals, the second variant, which is consist of serogroups of Leptospira: *Pomona, Tarassovi, Grippytyphosa, Sejroe*

(serovar *hardjo*). The antigen structure of the present vaccine does not response the etiological structure of leptospirosis in cattle which is in recent time that is why the usage of the VDNKI vaccine against leptospirosis of animals, the second variant, it is impossible to provide with the effective specific prophylaxis of leptospirosis of that species of animal.

Scientists of laboratory of Leptospirosis of the Institute of Veterinary Medicine has developed and manufactured one series of experimental multivalent vaccine for cattle, which consists of five serogroups of *Leptospira* (*Tarassovi*, *Grippotyphosa*, *Sejroe*, *Hebdomadis* та *Icterohaemorrhagiae*), which are currently the most widespread on the territory of Ukraine.

In previous studies have examined the following indicators vaccine: pH, sterility, residual amount inactivant, complete inactivation, harmlessness and immunogenic activity (in laboratory animals - rabbits) [8]. The results of the studies found that all three vaccine series satisfy the necessary regulatory requirements, and this vaccine can be tested on farm animals, so the next step of our research was to determine the optimal immunizing dose vaccine created in a production environment, to susceptible animals – cattle.

The research results will be the scientific basis for the development and implementation of new and effective means for the prevention of leptospirosis cattle in Ukraine.

The purpose of the study is to determine the optimal immunizing dose of concentrated inactivated polyvalent vaccine against leptospirosis in cattle.

Materials and research methods. To determine the optimal immunizing dose of inactivated polyvalent have created a vaccine against leptospirosis animal experiment was conducted on cattle.

Immunizing dose was determined for the two age groups of cattle: cattle and cattle under one year older than one year. First, research was conducted to determine the dose vaccine for cattle under one year. For this study, the principle of analogues was formed four groups of cattle (4 animals in the group aged 6-8 months).

For the purpose of the experiment cattle under one year vaccine was injected in doses of 1, 3 and 5 cm³. In a control using polyvalent vaccine against leptospirosis

VDNKI animals, the second option, which included the following serogroup leptospira: *Pomona*, *Tarassovi*, *Grippotyphosa*, *Sejroe* (serovar *hardjo*), which is used currently in Ukraine to prevent leptospirosis this type of animal. The vaccine is taken to control used in recommended doses for its application, namely cattle aged 6 to 8 months was dose – 4 cm³.

The next stage of this study was to determine the optimal immunizing dose vaccine created for cattle older than one year. For this study, as the principle of analogues were formed four groups of cattle older than one year (4 animals in the group aged 16-20 months).

For the purpose of the experiment subjects' cattle aged over one year injected vaccine at a dose of 3, 5 and 7 cm³. Polyvalent vaccine against leptospirosis VDNKI animals was used as a control in the second option. The vaccine is taken to control used in recommended doses for its application, namely cattle aged over one year was dose – 8 cm³.

Before carrying out research in all experimental cattle (both age groups) was selected and investigated in blood microagglutination test (MAT) for the presence of leptospirosis antibodies. When setting reaction using eight strains of leptospira diagnostic, they belong to eight serological groups' leptospira (*Australis*, *Canicola*, *Grippotiphosae*, *Hebdomadis*, *Icterohaemorrhagiae*, *Pomona*, *Sejroe*, *Tarassovi*).

Later after prophylactic vaccination in experimental animals blood samples were taken and treated serum. In the sera of immunized cattle antibody titer was determined in PMA, five times 14, 21, 60, 90 and 180 days after inoculation. Six leptospira vaccine strains have been used in MAT providing the manufacture of vaccines. Antibody titers were determined at PMA in 8 dilutions from 1:25 to 1: 3200 (multiplicity 2). That dilution of serum which was observed half and more leptospira agglutination, considered by antigen titer investigated.

1. List of industrial strains of leptospira used to manufacture vaccines against bovine leptospirosis

Item №	Serogroup	Serovar	Strain
1.	<i>Sejroe</i>	<i>polonica</i>	<i>493 Poland</i>
2.	<i>Sejroe</i>	<i>hardjo</i>	<i>Hardjoprajtno</i>
3.	<i>Hebdomadis</i>	<i>kabura</i>	<i>Kabura</i>
4.	<i>Grippotyphosa</i>	<i>grippotyphosa</i>	VGNKI-1
5.	<i>Tarassovi</i>	<i>tarassovi</i>	VGNKI -4
6.	<i>Icterohaemorrhagiae</i>	<i>icterohaemorrhagiae</i>	VGNKI -2

Results and discussion. During the experiment, all heads of cattle (flocks both age groups) after administration of the drug was observed immunobiological lack of common manifestations (depression, fever, anaphylaxis) and there were no local reactions.

Following immunization of cattle under one year experimental series polyvalent vaccine against leptospirosis animals we observed a strong induction of antibodies against serogroup leptospira, which were part of the vaccine, depending on dose vaccination (Table. 2, Fig. 1-6).

There was a general trend in all grafted doses of vaccine, namely antibody titer in MAT dramatically increased on the 14th day and reached maximum performance on 21st day after vaccination, and then on the 60th day he sharply declined. We believe the sharp decline in antibody titers to the 60th day after vaccination due to the different classes of antibodies formed in response to the leptospirosis antigen. The reaction microagglutination determines immunoglobulin Ig M, and preventive activity immunoglobulin Ig G [3].

2. Antibody titers at MAT 14, 21, 60, 90 and 180 days on different serogroup leptospira bovine serum (aged less than one year) vaccinated different doses of the vaccine - 1, 3 and 5 cm³, (M±m, n=24)

Dose	Serogroup	The titer of antibodies on days after vaccination				
		14	21	60	90	180
1 cm ³	<i>Sejroe (polonica)</i>	1:93,7	1:100	1:31,2	1:25	1:12,5
	<i>Sejroe (hardjo)</i>	1:62,5	1:68,7	1:43,7	1:18,7	1:6,2
	<i>Hebdomadis</i>	1:93,7	1:125	1:37,5	1:31,2	1:18,7
	<i>Icterohaemorrhagiae</i>	1:43,7	1:75	1:25	1:12,5	1:6,2
	<i>Grippotyphosa</i>	1:43,7	1:62,5	1:18,7	1:12,5	1:6,2
	<i>Tarassovi</i>	1:56,2	1:93,7	1:18,7	1:18,7	1:12,5
	Average titer	1:65,6±7,8***	1:87,5±7,8***	1:29,1±3,5** *	1:19,8±2,3* **	1:10,4±1,7*
3 cm ³	<i>Sejroe (polonica)</i>	1:300	1:400	1:100	1:56,2	1:31,2
	<i>Sejroe (hardjo)</i>	1:225	1:300	1:75	1:62,5	1:31,2
	<i>Hebdomadis</i>	1:375	1:550	1:125	1:87,5	1:37,5
	<i>Icterohaemorrhagiae</i>	1:175	1:300	1:75	1:43,7	1:31,2
	<i>Grippotyphosa</i>	1:175	1:150	1:50	1:37,5	1:12,5
	<i>Tarassovi</i>	1:275	1:225	1:62,5	1:43,7	1:18,7
	Average titer	1:254,2±26,1	1:320,8±43,0	1:81,2±8,7	1:55,2±5,7*	1:27,0±3,2*
5 cm ³	<i>Sejroe (polonica)</i>	1:350	1:450	1:87,5	1:50	1:37,5
	<i>Sejroe (hardjo)</i>	1:275	1:350	1:62,5	1:56,2	1:31,2
	<i>Hebdomadis</i>	1:425	1:500	1:125	1:112,5	1:43,7
	<i>Icterohaemorrhagiae</i>	1:175	1:350	1:87,5	1:50	1:31,2
	<i>Grippotyphosa</i>	1:225	1:150	1:43,7	1:43,7	1:6,25
	<i>Tarassovi</i>	1:300	1:200	1:56,2	1:37,5	1:12,5
	Average titer	1:291,7±27,9* *	1:333,3±44,1*	1:77,1±9,6	1:58,3±7,5*	1:27,0±4,9
Contro	<i>Pomona</i>	1:175	1:275	1:100	1:50	1:25
	<i>Tarassovi</i>	1:250	1:225	1:62,5	1:31,2	1:12,5

<i>Grippotyphosa</i>	1:150	1:175	1:43,7	1:37,5	1:12,5
<i>Sejroe (hardjo)</i>	1:250	1:275	1:62,5	1:43,7	1:18,7
Average titer	1:206,2±18,3	1:237,5±15,7	1:67,2±6,9	1:40,6±2,6	1:17,2±1,9

Remark: * - significant difference relatively to control $p \leq 0,05$;
 ** - significant difference relatively to control $p \leq 0,01$;
 *** - significant difference relatively to control $p \leq 0,001$.

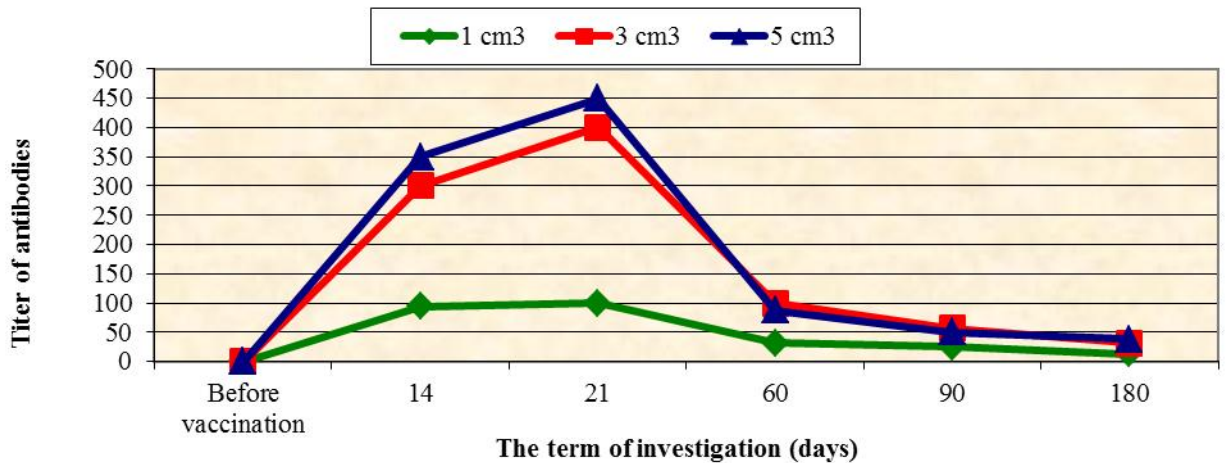


Fig. 1 Antibody titers to serogroup *Sejroe (polonica)* in bovine serum (aged less than one year) vaccinated with different doses of the vaccine (n = 4)

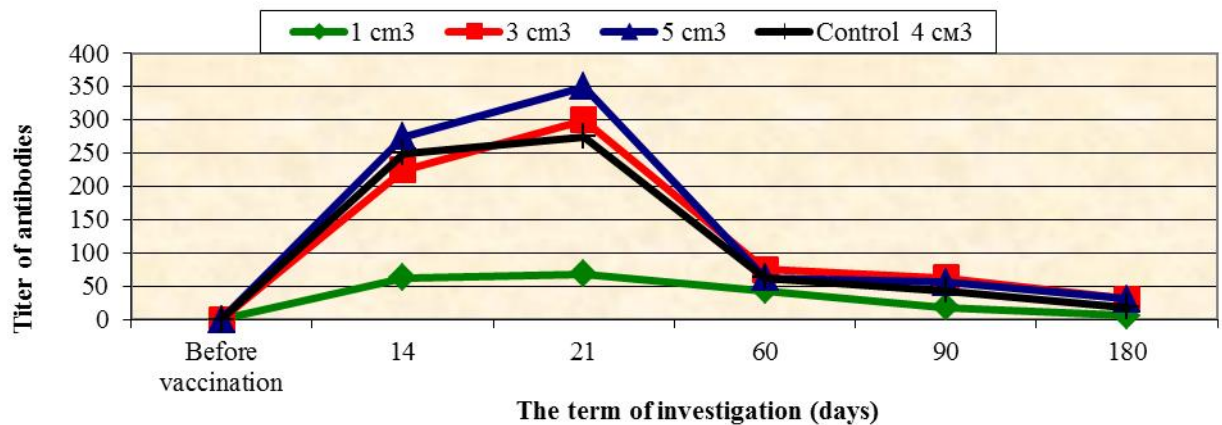


Fig. 2 Antibody titers to serogroup *Sejroe (hardjo)* in bovine serum (aged less than one year) vaccinated with different doses of the vaccine (n = 4)

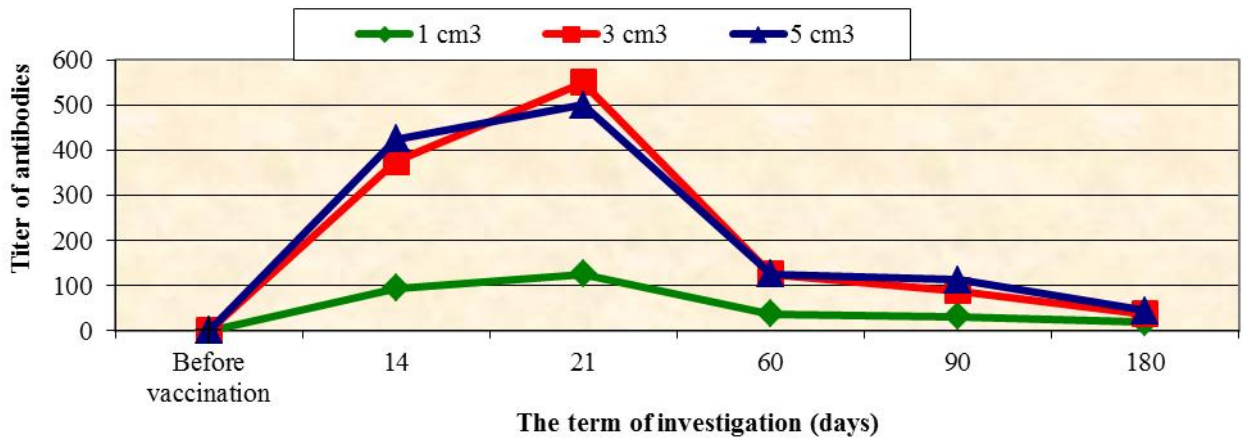


Fig. 3 Antibody titers to serogroup *Hebdomadis* in bovine serum (aged less than one year) vaccinated with different doses of the vaccine (n = 4)

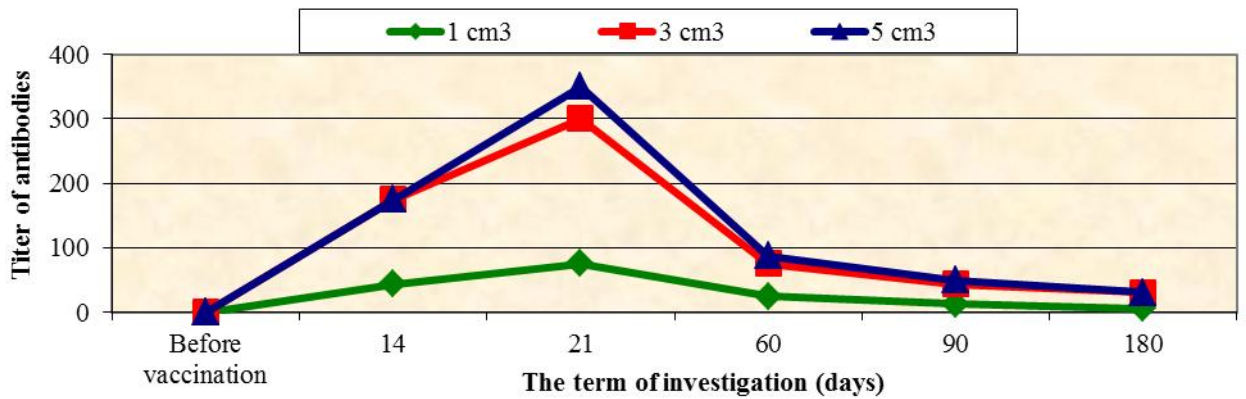


Fig. 4 Antibody titers to serogroup *Icterohaemorrhagiae* in bovine serum (aged less than one year) vaccinated with different doses of the vaccine (n = 4)

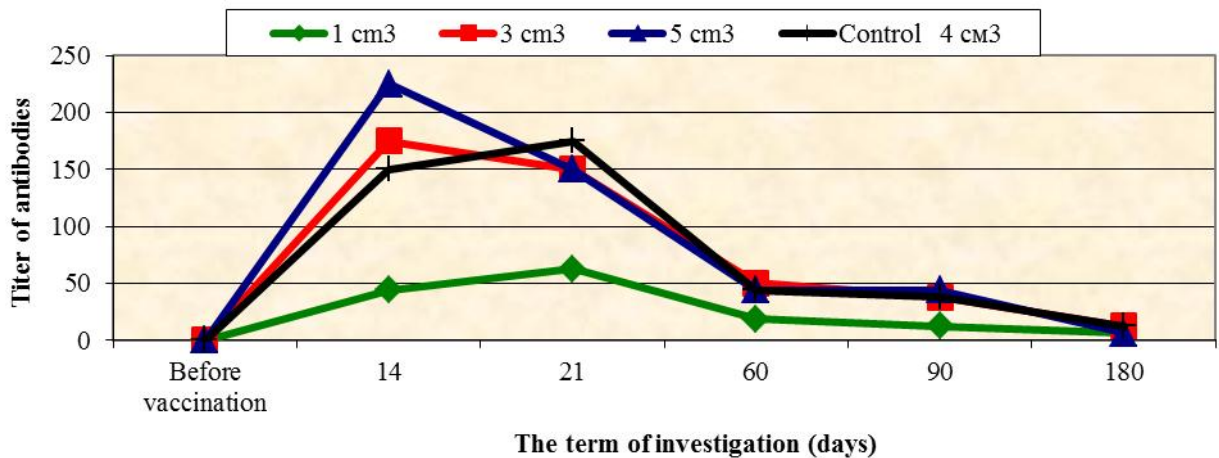


Fig. 5 Antibody titers to serogroup *Grippotyphosa* in bovine serum (aged less than one year) vaccinated with different doses of the vaccine (n = 4)

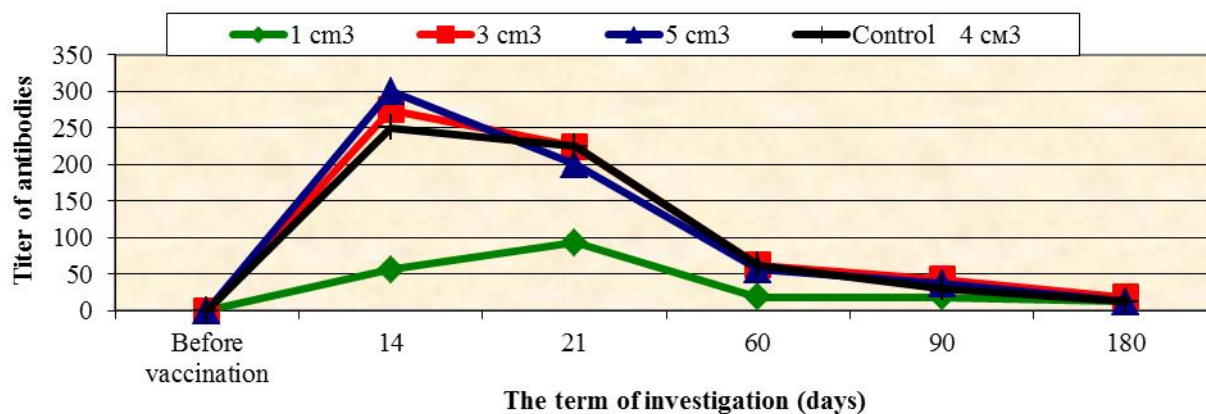


Fig. 6 Antibody titers to serogroup *Tarassovi* in bovine serum (aged less than one year) vaccinated with different doses of the vaccine (n = 4)

Judging by the formation of specific antibodies (Fig. 1-6) in cattle vaccinated at a dose of 3 cm³ and 5 cm³ immunity was formed about the same intensity for all serogroup of *Leptospira*, which were part of a series of experimental vaccines and exceeded or was at a similar level of performance in group of animals vaccinated VDNKI polyvalent vaccines against leptospirosis.

Specific antibodies in cattle vaccinated at a dose of 1 cm³ not provide immunity formation of high tension and were significantly lower than titers VDNKI vaccine, which was taken as control.

The next stage of this study was to determine the optimal immunizing dose vaccine created for cattle older than one year. Indicators of immunogenic activities of a series of experimental vaccines in cattle in this age group are shown in Table.3 and Fig.7-12.

In cattle aged over year before vaccination were found low titers of antibodies to *Leptospira* serogroup *Sejroe*, *Hebdomadis Icterohaemorrhagiae* and *Grippotyphosa* (1: 4,7 ± 1,9 to 1: 10,4 ± 2,0), which is associated with their prior vaccination (vaccination of animals of this group was held for 7 months before the experiment).

According to research results (Table. 3), bovine animals over one year observed the same trend as in cattle under one year, namely at all doses of vaccine grafted antibody titer in MAT dramatically increased by the 14th day and reaches maximum performance on 21st day of vaccination, then the 60th day it has been sharply

declined.

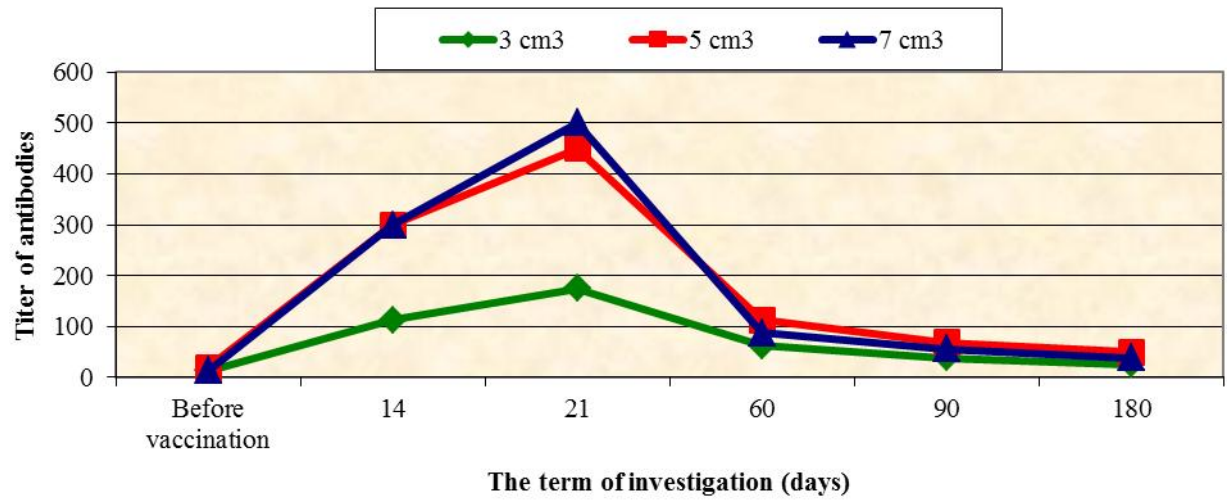


Fig. 7 Antibody titers to serogroup *Sejroe (polonica)* in bovine serum (older than one year) vaccinated with different doses of the vaccine (n=4)

3. Antibody titers at MAT 14, 21, 60, 90 and 180 days on different serogroup leptospira bovine serum (older than one year) vaccinated different doses of the vaccine - 3, 5 and 7 cm³, (M±m, n=24)

Dose	Serogroup	Antibody titers before vaccinations	The titer of antibodies on days after vaccination				
			14	21	60	90	180
3 cm ³	<i>Sejroe (polonica)</i>	1:12,5	1:112,5	1:175	1:62,5	1:37,5	1:25
	<i>Sejroe (hardjo)</i>	1:12,5	1:87,5	1:137,5	1:43,7	1:25	1:18,7
	<i>Hebdomadis</i>	1:6,2	1:100	1:137,5	1:43,7	1:25	1:31,2
	<i>Icterohaemorrhagiae</i>	1:6,2	1:50	1:62,5	1:37,5	1:31,2	1:12,5
	<i>Grippotyphosa</i>	1:18,7	1:56,25	1:68,7	1:31,2	1:12,5	1:6,2
	<i>Tarassovi</i>	0	50	1:100	1:37,5	1:25	1:18,7
	Average titer	1:9,4±2,2	1:76,0±10,0***	1:113,5±15,2**	1:42,7±3,0***	1:26,0±2,3***	1:18,7±2,6
5 cm ³	<i>Sejroe (polonica)</i>	1:18,7	1:300	1:450	1:112,5	1:68,7	1:50
	<i>Sejroe (hardjo)</i>	1:12,5	1:250	1:375	1:87,5	1:75	1:37,5
	<i>Hebdomadis</i>	1:6,2	1:500	1:600	1:150	1:106,2	1:37,5
	<i>Icterohaemorrhagiae</i>	0	1:300	1:375	1:100	1:68,7	1:31,2
	<i>Grippotyphosa</i>	1:18,7	1:225	1:175	1:62,5	1:37,5	1:18,7
	<i>Tarassovi</i>	0	1:225	1:225	1:56,2	1:50	1:12,5
	Average titer	1:9,4±3,0	1:300±27,9**	1:366,7±46,4***	1:94,8±10,9	1:67,7±6,7	1:31,2±4,3
7 cm ³	<i>Sejroe (polonica)</i>	1:12,5	1:300	1:500	1:87,5	1:56,2	1:37,5
	<i>Sejroe (hardjo)</i>	1:18,7	1:300	1:375	1:100	1:75	1:43,7
	<i>Hebdomadis</i>	1:12,5	1:450	1:650	1:162,5	1:87,5	1:37,5

	<i>Icterohaemorrhagiae</i>	0	1:350	1:300	1:75	1:56,2	1:18,7
	<i>Grippotyphosa</i>	1:12,5	1:250	1:225	1:62,5	1:31,2	1:12,5
	<i>Tarassovi</i>	1:6,2	1:225	1:250	1:62,5	1:37,5	1:18,7
	Average titer	1:10,4±2,0	1:312,5±24,4***	1:383,3±53,4**	1:91,7±11,0	1:57,3±6,7	1:28,1±4,8
Control	<i>Pomona</i>	0	1:175	1:175	1:87,5	1:37,5	1:18,7
	<i>Tarassovi</i>	0	1:150	1:175	1:87,5	1:56,2	1:25
	<i>Grippotyphosa</i>	1:6,2	1:200	1:125	1:100	1:37,5	1:12,5
	<i>Sejroe (hardjo)</i>	1:12,5	1:250	1:275	1:125	1:75	1:37,5
	Average titer	1:4,7±1,9	1:193,7±13,1	1:187,5±20,9	1:100±5,2	1:51,5±5,9	1:23,4±3,3

Remark: * - significant difference relatively to control $p \leq 0,05$;
 ** - significant difference relatively to control $p \leq 0,01$;
 *** - significant difference relatively to control $p \leq 0,001$.

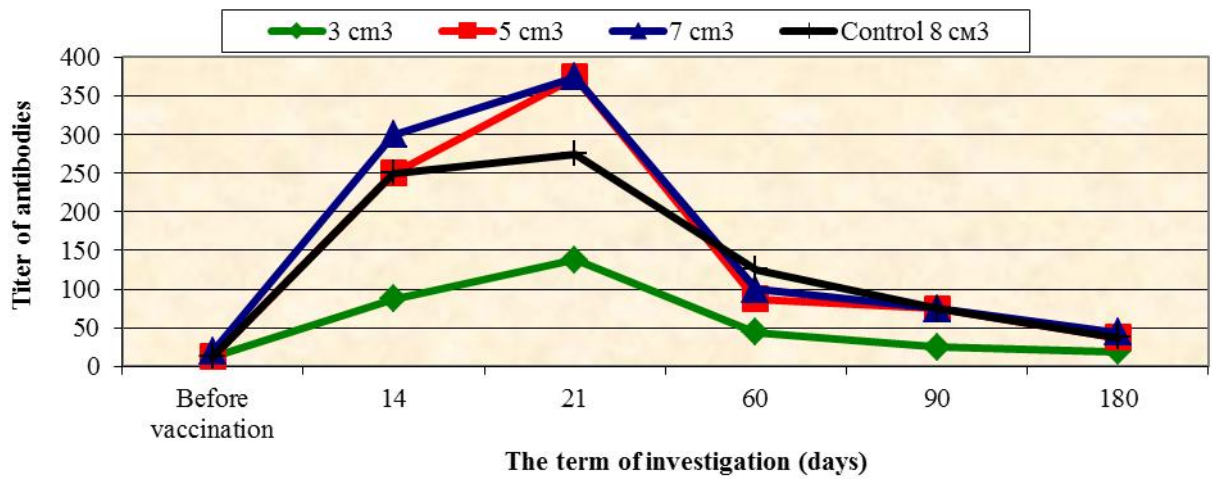


Fig. 8 Antibody titers to serogroup *Sejroe (hardjo)* in bovine serum (older than one year) vaccinated with different doses of the vaccine (n = 4)

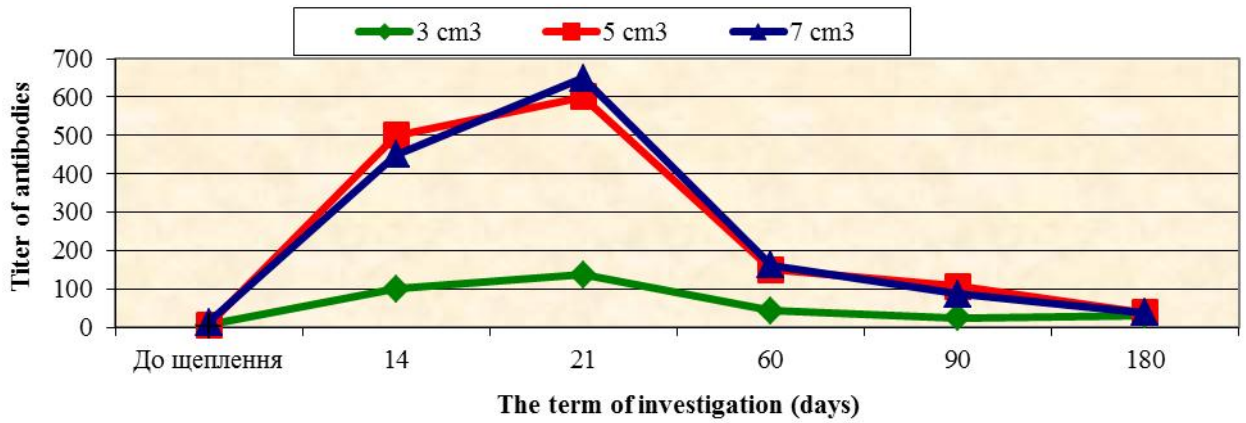


Fig. 9 Antibody titers to serogroup *Hebdomadis* in bovine serum (older than one year) vaccinated with different doses of the vaccine (n = 4)

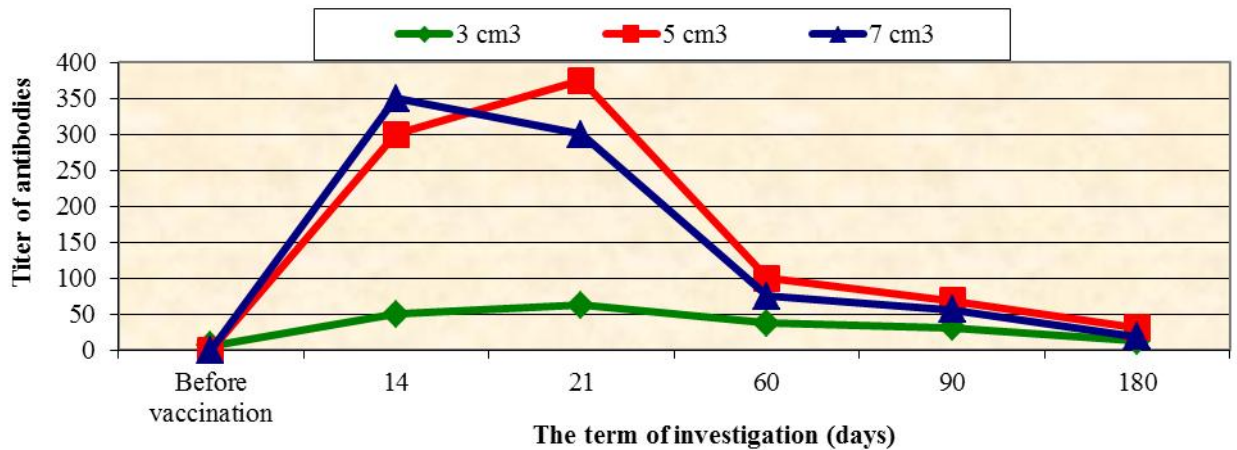


Fig. 10 Antibody titers to serogroup *Icterohaemorrhagiae* in bovine serum (older than one year) vaccinated with different doses of the vaccine (n = 4)

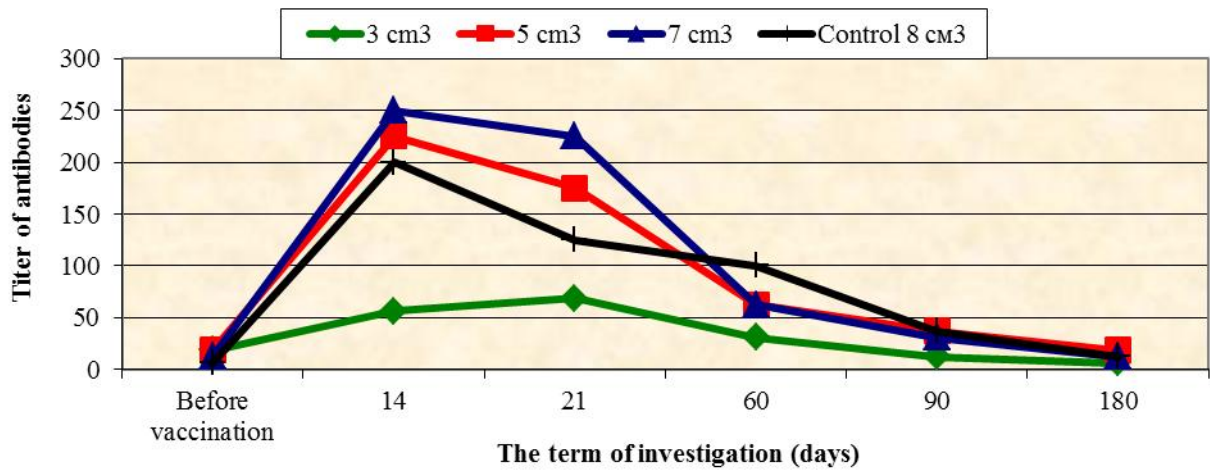


Fig. 11 Antibody titers to serogroup *Grippotyphosa* in bovine serum (older than one year) vaccinated with different doses of the vaccine (n = 4)

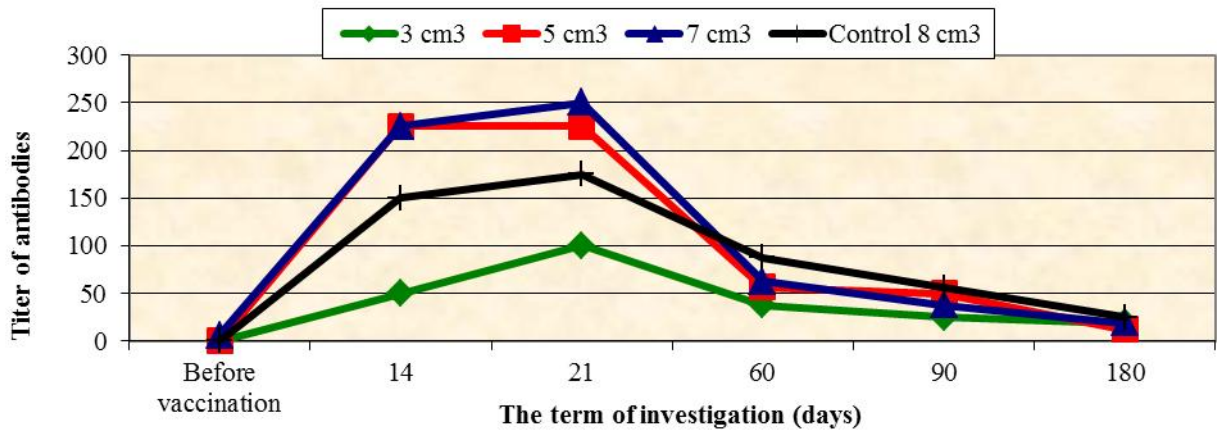


Fig. 12 Antibody titers to serogroup *Tarassovi* in bovine serum (older than one year) vaccinated with different doses of the vaccine (n = 4)

It was established that in dairy herd cattle according to the immunological studies (the age more than one year), injected in the dose 5 cm³ and 7 cm³, the immunity was received to all serogroups of *Leptospira*, which was in the experimental series of the vaccine. Immunological characteristics to the respective research groups in cattle extremely exceeded the analogical characteristics of the control group of animals, injected with the polyvalent vaccine VDNKI against leptospirosis. The rates of antibodies' titers to serogroups *Sejroe*, *Icterohaemorrhagiae*, *Tarassovi* and *Grippotyphosa* were variated with inconsiderable differences. But, it should be admitted, that the rates of antibodies'

titers to serogroups of *Leptospira Hebdomadis* (Fig.9), were significantly higher than the rates of the other four serogroups of *Leptospira*.

It was established according to the immunological studies, that the injection dose 3 cm³ for the cattle under the age older than one year didn't provided with the immunity of the high tension, and the rates of the antibodies titers in MAT were significantly lower for similar rates in cattle, immunized with the vaccine VDNKI, taken as the control.

According to the studies it was established, that the optimum dose of the injection for the dairy herd cattle (the age more than one year) is 5 cm³.

It was established according to the results of immunological studies in first year cattle the rates of antibodies' titers in MAT were lower than the titers of the age more than one year cattle.

According to some authors the immune response of the animal (revaccination) is different for the first and the second injection of the antigen. Revaccination causes generally more intensive and high making of specific antibodies. It is obvious that the rates of antibodies titers in dairy herd cattle were higher because of the previous vaccination, before six months before the beginning of the research, in the same time, as the immunity of the one year cattle was formed in the response to the first injection of the antigen.

It were not fixed during the research period none of the investigated animals (of the both age groups) with clinical signs that are specific for leptospirosis and extremely exceeding of the antibodies' titers in MAT that has been specified the generation of the disease.

Conclusions

The results of our studies identified the optimal dose of the experimental vaccine against leptospirosis in cattle, which provide immunity maximum strength and durability. Doses are: for cattle under 1 year – 3 cm³, for cattle aged 1 year or more – 5 cm³.

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ВИЗНАЧЕННЯ ОПТИМАЛЬНОЇ ІМУНІЗУЮЧОЇ ДОЗИ КОНЦЕНТРОВАНОЇ ПОЛІВАЛЕНТНОЇ ІНАКТИВОВАНОЇ ВАКЦИНИ ПРОТИ ЛЕПТОСПИРОЗУ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ

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*Ученими лабораторії лептоспірозу Інституту ветеринарної медицини розроблена та виготовлена експериментальна серія полівалентної вакцини проти лептоспірозу для великої рогатої худоби, до складу якої входять п'ять серогруп лептоспір (*Icterohaemorrhagiae*, *Tarassovi*, *Hebdomadis*, *Sejroe*, *Grippotyphosa*), які на сьогодні найбільш поширені на території України. Наведено дані щодо визначення оптимальної імунізуючої дози експериментальної серії інактивованої полівалентної вакцини проти лептоспірозу великої рогатої худоби. Антитілоутворення визначали в реакції мікроаглютинації у динаміці через 14, 21, 60, 90 та 180 діб після вакцинації.*

Ключові слова: *вакцина, лептоспіра, лептоспіроз, велика рогата худоба, серогрупа, штам, антитіла, реакція мікроаглютинації*

ОПРЕДЕЛЕНИЕ ОПТИМАЛЬНОЙ ИММУНИЗИРУЮЩЕЙ ДОЗЫ КОНЦЕНТРИРОВАННОЙ ПОЛИВАЛЕНТНОЙ ИНАКТИВИРОВАННОЙ ВАКЦИНЫ ПРОТИВ ЛЕПТОСПИРОЗА КРУПНОГО РОГАТОГО СКОТА

В. В. Уховский

*Учеными лаборатории лептоспироза Института ветеринарной медицины разработана и изготовлена экспериментальная серия поливалентной вакцины против лептоспироза крупного рогатого скота, в состав которой входят пять серогрупп лептоспир (*Icterohaemorrhagiae*, *Tarassovi*, *Hebdomadis*, *Sejroe*, *Grippotyphosa*), которые в настоящее время наиболее распространены на территории Украины. Представлены данные по определению оптимальных*

иммунизирующих доз экспериментальной серии инактивированной поливалентной вакцины против лептоспироза крупного рогатого скота. Антителообразование определяли в реакции микроагглютинации в динамике через 14, 21, 60, 90 и 180 суток после вакцинации.

Ключевые слова: вакцина, лептоспира, лептоспироз, крупный рогатый скот, серогруппа, штамм, антитела, реакция микроагглютинации