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STUDY OF IMMUNOGENIC PROPERTIES OF EXPERIMENTAL SERIES OF BIVALENT VACCINE AGAINST CARNIVOROUS LEPTOSPIROSIS IN LABORATORY CONDITIONS

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Summary. Leptospirosis is an infectious natural-focal disease of many species of animals and human with a wide range of clinical signs. The most common serovars causing leptospirosis in dogs are Icterohaemorrhagiae and Canicola. Analyzing the epizootic situation concerning leptospirosis of carnivores in Ukraine, employees of the Laboratory of Leptospirosis of Institute of Veterinary Medicine of the National Academy of Agrarian Sciences have developed and produced successively three series of bivalent inactivated vaccine against leptospirosis of carnivores, taking into account the etiological structure of the disease of this animal species. The paper presents the results of the study of three experimental series of inactivated polyvalent vaccine against leptospirosis of carnivores regarding: pH, sterility, residual amounts of inactivant, completeness of inactivation, harmlessness and immunogenic activity. It has been established that according to the indicators of sterility, completeness of inactivation, residual amount of inactivant, harmlessness and concentration of hydrogen ions, all three series of vaccine meet the requirements and norms of normative documentation. Intravenous administration of all three experimental series of the vaccine to the experimental rabbits provided the formation of specific anti-leptospirosis antibodies in the titers corresponding to the parameters of the immunogenicity norms established in the technical conditions of the drug.

Keywords: vaccine, leptospirosis, Icterohaemorrhagiae, Canicola, dogs, rabbits, microscopic agglutination test

Introduction. Leptospirosis is a zoo-anthroponotic natural-focal infection characterized by short-term fever, anemia, jaundice, necrosis of the mucous membranes and skin, hemoglobinuria, atony of the gastrointestinal tract and weight loss, abortions and the birth of an unviable offspring (Nedosiekov, Ukhovskyi and Kucheriavenko, 2011). One of the main measures to control leptospirosis is vaccination. The vaccine against leptospirosis is used for the active immunization of animals. It prevents the acute course of the disease, the death of animals, abortions, carriage of *Leptospira* (Adler, 2015; Balks et al., 2013; Walker and Srinivas, 2013).

The biological industry of Ukraine does not produce domestic vaccine against leptospirosis of carnivores, and uses only foreign vaccines for the prevention of carnivorous leptospirosis, therefore we have carried out scientific researches on perfection and development of a new technology for production of immunoprophylactic agent against leptospirosis of carnivores.

The employees of the Laboratory of Leptospirosis of Institute of Veterinary Medicine of the National Academy of Agrarian Sciences have developed and produced sequentially three series of bivalent inactivated vaccine against leptospirosis of carnivores taking into account the etiological structure of the disease of this species of animals.

The technical result of the designed vaccine is: increased immunogenic and antigenic activity of selective strains (registration numbers provided by the Depository of the State Scientific and Control Institute of Biotechnology and Strains of Microorganisms 354 and 360) used for the production of the vaccine; reduced immunization dose of the vaccine due to the modern methods of concentration with polyethylene glycol.

Leptospirosis is one of the most prevalent antropozoonotic infections in many countries of the world and in particular in Ukraine (Ukhovskyi et al., 2018). To date, there are over 250 leptospira serovars in 26 serogroups (Walker and Srinivas, 2013). Animals of different species suffer from leptospirosis: cattle, pigs, horses, sheep, goats, deer, foxes, arctic foxes, mink, dogs, etc. (Pyskun et al., 2018; Stepna et al., 2016; Ukhovskyi et al., 2014). The most common serovars causing leptospirosis in dogs before the introduction of leptospirosis vaccine 30 years ago were Canicola and Icterohaemorrhagiae. After the production of the bivalent vaccine, other strains have become more common, including Grippotyphosa, Pomona, Bratislava, and Autumnalis (Alton et al., 2009). This may be the result of an increasing number of contacts between dogs and reservoir pathogen hosts (Martin et al., 2014). The selection of leptospira serovars for vaccine development is a very important step. For the most effective prevention, you need to use only those serovars that circulate in this region.

At present, the most common leptospira, which cause leptospirosis disease among carnivores in Ukraine, are leptospira of the following serogroups: Canicola and Icterohaemorrhagiae (Babyuk et al., 2009).

The aim of the work was to study the properties of three, produced sequentially, experimental series of polyvalent vaccines in laboratory animals, to determine the following parameters: pH, sterility, residual amount of inactivant, completeness of inactivation, harmlessness and immunogenic activity.

Materials and methods. Two leptospira strains, Icterohaemorrhagiae and Canicola, were used in the production of three series of experimental bivalent vaccines. The list of these strains is shown in the Table 1. All the series of these vaccines were made from the same leptospira strains, and by the same technology.

Table 1 — List of strains used in the manufacture of the vaccine

No.	Serogroup	Serovar	Strain	Registration number
1	Ictero-haemorrhagiae	Ictero-haemorrhagiae	VGNKI-2	354
2	Canicola	Canicola	VGNKI-3	360

The leptospira strains used to make the vaccine, were cultured on a Kortgof medium with the addition of 10% of sheep blood serum at 27–28 °C in a dark room.

For the manufacture of vaccines there were used cultures with accumulation of at least 75 million leptospira in one centimeter cubic, that is, not less than 60 leptospira in the field of view of the microscope.

Each leptospira serogroup was cultured separately, then they were poured into one container and inactivated.

Inactivation of bacteria was carried out with a solution of phenol, it was added to the culture in the amount of 0.5% to the volume of the vaccine. The preserved culture was maintained at 27–28 °C for 12 hours, this was followed by microscopy of the culture in a dark field of the microscope. To concentrate the vaccine, a mixture of leptospira cultures in a bottle was precipitated by the addition of a solution of polyethylene glycol (PEG 6000) of 10–12% to the volume of the preparation. It was previously prepared sterile mother liquor of PEG — 70%. After that, the vaccine was mixed vigorously with a stirrer for 10–15 minutes. Additionally, the preparation was concentrated by removing 50% of the supernatant from a mixture of cultures that were inactivated and precipitated.

After adding the PEG from the bottle, samples were taken and checked for sterility. Sterility of the vaccine was determined according to DSTU 4483:2005 (DSSU, 2005).

The concentration of hydrogen ions was determined by a pH meter in accordance with the instructions for its use. Determination of the residual amount of inactivant was carried out in accordance with the Guidelines 4.1/4.2.588-96.

To determine the completeness of inactivation of the vaccine, we conducted three consecutive passages of it on the Kortgof medium with addition of 10% rabbit serum. In this case, three test tubes were used for each passage for samples from each vial taken for control. The presence of live leptospira in the field of view of the microscope was determined by microscopy in a dark field (an increase of 20×10×1.5 or 20×15).

The harmlessness of the experimental series of vaccines was determined on white mice with body weight from 18 to 20 g, 10 mice for testing of each series.

Vaccinations were carried out by subcutaneous administration of vaccines at a dose of 0.3 cm^3. The injection site was treated with 70% ethyl alcohol. Experimental animals were observed for 10 days to determine their general state. Each animal was used once.

The antigenic properties of the vaccine were determined on rabbits with body weight of 3.0–3.5 kg, five animals for testing of each vaccine series.

After shaking, 10 cm^3 of vaccine were taken with the sterile pipette from each of five vials with one series of vaccine, and transferred to a sterile vial of 100 cm^3. The resulting average sample of the vaccine was shaken and administered intravenously at a dose of 0.75 cm^3 to five rabbits. 25 days later, blood samples were taken from vaccinated rabbits and examined in the microscopic agglutination test. Similar studies have been conducted with all three series of vaccines.

Blood serum of rabbits was investigated in the microscopic agglutination test with the strains of the corresponding leptospira serovars, which were part of the tested series of vaccines.

The antibody titers in the indicated test were determined in 6 dilutions from 1:50 to 1:1,600 (multiplicity of 2). Solution of serum, in which half and more agglutination of leptospira was observed, was considered as a titer of antigen.

Experimental data was calculated statistically (Lakin, 1990).

Results. All series of the drug in appearance and by color were homogeneous grayish-white liquid with a slight precipitate, which, when shaking, easily broke down to the formation of a homogeneous suspension.

The results of the study of vaccine series by the parameters of sterility, completeness of inactivation, residual amount of inactivant (phenol), harmlessness and concentration of hydrogen ions are presented in the Table 2. According to the requirements of the regulatory documents, the concentration of hydrogen ions in the finished preparation should be within the range of 7.2–7.4. According to the data of the Table 2, the concentration of hydrogen ions in the experiment varied in the range 7.24–7.36, which corresponds to the norm. Testing the preparation for sterility, according to DSTU 4483:2005 (DSSU, 2005), showed that all series of vaccines were sterile.

During the three times passage of the vaccine on a nutrient medium for the growth of leptospira, no growth of live spirochaeta was observed, indicating complete inactivation of the antigen in the tested vaccine.

The residual amount of inactivant in the vaccine ranged from 0.41 to 0.48, that is, within the permissible concentrations, which should not exceed 0.5% according to the norm.

Table 2 — Physical and biological characteristics of experimental series of vaccine against leptospirosis of carnivores

Characteristics	Series 1	Series 2	Series 3
pH of vaccine	7.24	7.36	7.28
Sterility	sterile		
Completeness of inactivation	At three-time passage of the vaccine on the nutrient medium there was no growth of leptospira		
Harmlessness	harmless		
Residual amount of inactivator (phenol), %	0.48	0.41	0.47

When determining the harmlessness of the vaccine, by administering the drug to laboratory animals, it has been established that during the observation period all the animals remained alive without any local and symptomatic manifestations caused by the vaccine. The vaccine was considered harmless.

Thus, by the characteristics of sterility, completeness of inactivation, residual amount of inactivant, harmlessness and concentration of hydrogen ions, all three series of vaccine met the requirements and norms of normative documentation.

When determining the indicators of the immunogenicity of the produced drug, we have, based on the principle of analogues, formed three groups of rabbits (5 animals in the group) which were given a single intravenous administration of the vaccine at a dose of 0.75 cm^3. On the 25th day, the blood was taken from the vein of vaccinated rabbits. The blood was examined in the microscopic agglutination test. The results obtained are presented in the Table 3.

Before conducting a study of the vaccine immunogenicity, blood was collected from all experimental rabbits, and tested in the microscopic agglutination test for the presence of antibodies to pathogenic leptospira. Eight diagnostic strains of leptospira were used for the test, they belong to the eight serological groups Icterohaemorrhagiae, Australis, Pomona, Canicola, Sejroe, Hebdomadis, Grippotiphosa, Tarassovi. For all rabbits, the test was negative.

According to the requirements of normative and technological documentation, the vaccine is considered to be active, if not less than in four of the five vaccinated rabbits, the titer of antibodies in blood serum to Icterohaemorrhagiae and Canicola serogroups will be no less than 1:100.

The results of the studies indicate that all series of experimental vaccines were immunogenic and consistent with the indicators of immunogenicity standards set out in the technical specifications for this preparation.

As can be seen from the Table 3, the administration of all three experimental series of the vaccine into experimental rabbits provided the formation of specific anti-leptospirosis antibodies.

Table 3 — Titer of antibodies in the microscopic agglutination test for different leptospira serogroups in blood serum of vaccinated rabbits on the 25th day after vaccination

Series	Serial numbers of rabbits	Titers of antibodies to leptospira serogroups	
		Ictero-haemorrhagiae	Canicola
Series 1	Rabbit 1	1:1,600	1:1,600
	Rabbit 2	1:800	1:200
	Rabbit 3	1:800	1:800
	Rabbit 4	1:1,600	1:800
	Rabbit 5	1:800	1:800
	Average titer	1:1,120 ± 384	1:840 ± 304
Series 2	Rabbit 1	1:600	1:800
	Rabbit 2	1:200	1:800
	Rabbit 3	1:600	1:600
	Rabbit 4	1:800	1:200
	Rabbit 5	1:600	1:200
	Average titer	1:580 ± 206	1:520 ± 190
Series 3	Rabbit 1	1:1,600	1:1,600
	Rabbit 2	1:800	1:200
	Rabbit 3	1:200	1:800
	Rabbit 4	1:1,600	1:200
	Rabbit 5	1:1,600	1:200
	Average titer	1:1,160 ± 390	1:600 ± 195

Thus, the manufactured preparations are immunogenic and meet the requirements of the technical conditions for this immunobiological preparation.

According to the results of the conducted research, the 'Certificate of analysis of successively produced 3 series of vaccine' was drawn up and submitted to the State Research and Control Institute of Biotechnology and Strains of Microorganisms.

Conclusions. 1. When studying three experimental series of bivalent inactivated vaccine against leptospirosis in animals, it has been found that all of them were harmless to mice at a single subcutaneous administration at a dose of 0.3 cm^3.

2. The titers of antibodies in vaccinated with the experimental series of vaccine animals met the requirements of the technical conditions for this product.

3. According to the results of the research, it has been found that all three vaccine series met the necessary requirements, and this vaccine could be tested on carnivores.

Further research will be continued to study the effectiveness of the created bivalent vaccine in carnivorous animals.

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