

ANNOTATION
of the dissertation work by Madiana Orynbassar
on the topic «Subunit vaccine against COVID-19 for domestic cats: obtaining
and studying immunobiological properties» submitted for the degree of
Doctor of Philosophy (PhD) in the educational program (specialty) 8D09101 –
«Veterinary Medicine»

Relevance of the research topic. In the context of the COVID-19 pandemic, the transmission of SARS-CoV-2 from humans to animals, from animals to animals, and reverse transmission is of particular concern to the scientific community. Domestic cats are of special epidemiological significance, as they are in close contact with humans and exhibit high susceptibility to viral infection. SARS-CoV-2 was first identified in late 2019 and rapidly acquired the status of a global pandemic. Initially, it was assumed that the virus circulated exclusively among humans; however, subsequent studies have demonstrated its zoonotic nature and confirmed the possibility of human-to-animal transmission. Among domestic animals, cats are among the most susceptible to SARS-CoV-2. They not only become infected with the virus but are also capable of transmitting it to other cats. In most cases, infection in cats is asymptomatic or mild; in rare instances, respiratory disorders, decreased appetite, and lethargy are observed. However, asymptomatic carriage poses a particular epidemiological risk, as it contributes to the silent spread of infection. According to international data, the basic reproduction number (R_0) in cats exceeds 3, and the ability to shed the virus for prolonged periods may turn them into a natural reservoir of the pathogen. SARS-CoV-2 infection in members of the family Felidae has been officially documented in Asia, Europe, and the Americas. In this regard, within the framework of the One Health concept, vaccination of domestic cats is considered an integral component of comprehensive measures to counter the pandemic. The importance of this approach is supported by large-scale epidemiological studies at the international level. For instance, a study conducted in Chile in 2020–2024, analysing serum samples from 1,377 cats, revealed that the immune response persisted for up to 15 months. A study carried out in Wuhan, China, provided convincing evidence of SARS-CoV-2 transmission from humans to domestic cats. In this study, serum samples from 102 cats were tested. Antibodies to SARS-CoV-2 were detected in 15 animals (14.7%), of which 11 were confirmed to have virus-neutralising antibodies. These findings indicate that the cats had indeed been exposed to the virus and that their immune systems mounted a specific response.

Thus, the development of a safe and effective vaccine to protect domestic cats from SARS-CoV-2 is an urgent task for veterinary medicine and public health. Subunit vaccines occupy a special place in addressing this challenge. Subunit vaccines are a type of vaccine based on the administration of individual viral proteins (as a rule, the S protein), obtained through recombinant technology, in combination with an adjuvant. Compared with other types of vaccines, they possess a number of advantages: high safety, safety for animals with a weakened immune system, the possibility of repeated administration, and simple conditions of storage and

transportation. However, the immunogenicity of subunit vaccines may be low, which is due to weak stimulation of the immune system by purified proteins. Therefore, adjuvants play an important role in enhancing the immunogenicity of the vaccine.

Purpose of the dissertation research. Development and investigation of immunobiological properties of a safe, immunogenic, and effective subunit vaccine against COVID-19 for domestic cats.

Research objectives:

1. Development of the vaccine design and laboratory quality control of the pilot batch;
2. Comparative evaluation of the immunogenic and protective efficacy of various vaccine formulations in cats and determination of the optimal composition;
3. Assessment of the magnitude and duration of post-vaccination immunity in cats.

Research methods. Experimental studies were conducted at the BSL-3 and ABSL-3 laboratories of the National Scientific Center for Especially Dangerous Infections named after M.Aikimbayev. Permission to conduct research on the proposed project was obtained from the Institutional Committee for the Care and Use of Laboratory Animals (Protocol No. 4 dated 22 September 2020). In addition, research work was carried out at the International Center for Vaccinology at KazNARU.

The study employed a set of methods for determining the level of anti-Spike IgG by ELISA. Among all analytical methods, a special place is occupied by immunological analysis based on the use of enzymes as a label for the detection of antigen or antibody. The possibility of using enzymes as a label in immunoassay is обусловлена by the high catalytic activity of enzymes; with the aid of an appropriate substrate system, this enables the determination of enzyme concentration in solution at a level of 10^{-15} M.

All quantitative data obtained were processed using GraphPad Prism 9.0 software (GraphPad Software, USA). Antibody titers were calculated as geometric mean values (GMT).

Main provisions. Proven scientific hypotheses and other conclusions that constitute new knowledge:

1. Substantiation of the risk of SARS-CoV-2 spread among domestic cats and the establishment of a viral reservoir, which confirms the necessity of vaccinating cats as an integral component of the One Health strategy in the context of the COVID-19 pandemic;
2. Development of the design of a subunit vaccine against COVID-19 for domestic cats and laboratory quality control of the pilot batch, ensuring the safety, immunogenicity, and protective efficacy of the product;
3. Results of a comparative study of the immunogenicity and protective efficacy of various vaccine formulations in cats, conducted for the first time for a subunit vaccine intended for domestic cats.

Description of the main research results. In the course of the conducted research, the immunobiological properties of a subunit vaccine against COVID-19

for domestic cats were comprehensively studied, and the following key results were obtained:

1. A technology has been developed for producing a recombinant antigen based on the extracellular domain of the S protein of the SARS-CoV-2 virus. The extracellular domain of the S protein was obtained using *Trichoplusia ni* cells in a baculovirus expression system and purified by affinity chromatography. The identity of the obtained recombinant protein was confirmed by electrophoresis, Western blot, and mass spectrometric analysis. The purity of the protein exceeded 90%. The retention of the ability of the recombinant antigen to bind to the angiotensin-converting enzyme 2 receptor confirmed its biological activity.

2. Experimental formulations of a subunit vaccine against SARS-CoV-2 infection intended for domestic cats have been developed, and their main quality parameters have been determined. The vaccine samples contained 5 µg of the extracellular domain of the recombinant S protein and the oil-in-water emulsion adjuvant ESSAI O/W 1849102 or the aluminum hydroxide-based adjuvant Alhydrogel® 2%. The sterility of the finished vaccine samples was confirmed. The endotoxin content was less than 2 endotoxin units/mL, and the residual DNA content was less than 50 ng/mL.

3. Immunogenic activity of the vaccine. A pronounced effect of adjuvants on the immunogenic activity of the vaccine was established, and the optimal formulation was selected. After two intramuscular administrations of the vaccine with a 14-day interval, in the formulation with the ESSAI O/W 1849102 adjuvant, the geometric mean titer of immunoglobulin G antibodies against the S protein was 97,420. This indicator was approximately 2 times higher compared with the formulation containing the Alhydrogel® 2% adjuvant, and approximately 7 times higher compared with the antigen group without adjuvant. The geometric mean titer of neutralizing antibodies against the D614G variant was 945 in the ESSAI O/W 1849102 formulation, 450 in the Alhydrogel® 2% group, and 220 in the antigen group without adjuvant. Against the Delta variant, neutralizing antibodies were detected in all cats that received the vaccine with the ESSAI O/W 1849102 adjuvant, with a geometric mean titer of 315. In the Alhydrogel® 2% group, this indicator was 150, and in the antigen group without adjuvant — 106. Based on the obtained results, the composition based on 5 µg of the extracellular domain of the S protein and the oil-in-water emulsion adjuvant ESSAI O/W 1849102 was selected as the optimal experimental formulation of the NARUVAX-C19 (pets) vaccine in terms of immunogenic parameters.

4. Protective efficacy. In the controlled infection experiment, the protective efficacy of adjuvanted subunit vaccines, as well as their safety for domestic cats, was established. After intranasal infection with the SARS-CoV-2 virus, no infectious virus was isolated from the lung and heart tissues of the vaccine groups with the adjuvants ESSAI O/W 1849102 and Alhydrogel® 2%. In the formulation with the ESSAI O/W 1849102 adjuvant, the lowest level of viral load in the examined tissues was detected. This formulation was characterized by effective limitation of viral replication in the tissues of the lower respiratory tract and the heart. After vaccination, no clinically significant adverse reactions were observed.

5. As a result of the conducted research, a scientific and practical basis was formed for the development of a formulation of a recombinant subunit vaccine intended for the specific prevention of SARS-CoV-2 infection in domestic cats. The research results demonstrated the high immunogenic properties of the candidate vaccine NARUVAX-C19 (pets), created on the basis of 5 µg of the extracellular domain of the S protein and the oil-in-water emulsion adjuvant ESSAI O/W 1849102, its ability to form a neutralizing immune response against the original and Delta variants of the SARS-CoV-2 virus, as well as its protective effect under experimental conditions. The obtained results serve as a basis for further study of the duration of the vaccine's immune protection, long-term safety, impact on virus transmission, and the possibility of production on an industrial scale. The method of producing the vaccine is protected by patent of the Republic of Kazakhstan No. 35485.

Justification of the novelty and importance of the obtained results. For the first time, a subunit vaccine against COVID-19 for domestic cats has been developed. The technology for producing a subunit vaccine against SARS-CoV-2 intended for domestic cats has been established. For the first time, data on the immunogenicity and protective efficacy of various vaccine formulations in cats have been obtained. A comparative evaluation of the immunogenicity and protective efficacy of different adjuvant formulations of the subunit vaccine has been conducted in domestic cats. As a result of the study, the optimal type of adjuvant for cats was identified, and the mechanisms of immune response enhancement were characterised.

"A method for obtaining a subunit adjuvant vaccine for the specific prevention of SARS-CoV-2 infection in carnivores and other susceptible animal species. Patent No. 35485, issued by the Republican State Enterprise "National Institute of Intellectual Property" of the Ministry of Justice of the Republic of Kazakhstan on February 4, 2022."

Compliance with scientific development directions or state programs. "This dissertation research was conducted within the framework of the research project "A novel nanoparticle-based platform for an intranasal SARS-CoV-2 vaccine: design and testing in laboratory animal models." The work was financially supported by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP09259609)."

The relationship between the project objective and the content of the dissertation is as follows: while the project focused on developing a nanoparticle-based intranasal vaccine platform, the dissertation investigated the immunobiological properties of a subunit vaccine — thus, the two approaches complement each other, enabling a comprehensive investigation of vaccine immunity. Research within the project was conducted at the BSL-3 and ABSL-3 laboratories of the National Scientific Center for Especially Dangerous Infections named after M. Aikimbayev of the Republic of Kazakhstan, with strict adherence to high-level biosafety requirements. The results of the project served as the foundation for evaluating the protective efficacy of the vaccine in the dissertation work.

Description of the doctoral student's contribution to the preparation of each publication. The doctoral student took direct part in all stages of the dissertation research, including the formation of the scientific concept, the planning and execution of laboratory experiments, data collection and analysis, statistical processing of the results, interpretation of the obtained data, as well as the preparation and formalisation of scientific publications. The doctoral student mastered the safety instructions for working in BSL-3 and ABSL-3 laboratories and obtained the corresponding certificates. In the course of the research, the doctoral student conducted a comprehensive review of international scientific literature and compiled a bibliographic list consisting of 114 titles.

The following works have been published on the topic of the dissertation:

- 1 article in the international journal *Frontiers in Veterinary Science* (Q1), indexed in the Scopus and Web of Science databases;

- 1 patent for invention No. 35485 "Method for producing a subunit adjuvant vaccine for the specific prevention of SARS-CoV-2 infection in carnivores and other susceptible animals" has been obtained.

Volume and structure of the dissertation. The dissertation consists of 109 pages and includes an introduction, a literature review, materials and methods of the study, the results of original research, a conclusion, a list of references, and 5 appendices. The work contains 14 tables and 10 figures. The reference list comprises 114 sources.