

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.

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. Evaluation of the immunogenicity and protective efficacy of the vaccine in cats to select the optimal formulation;

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 KazNAU.

A comprehensive set of methods was employed in the work: the level of anti-Spike IgG was determined by ELISA (enzyme-linked immunosorbent assay). Among all analytical methods, immunological assays based on the use of enzymes as labels for the detection of antigens or antibodies occupy a special place. The feasibility of employing enzymes as labels in immunological analysis is attributed to their high catalytic activity, which, through the use of an appropriate substrate system, makes it possible to determine enzyme concentrations in solution at the level of 10^{-15} M.

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. Vaccine design and quality control. A subunit vaccine construct based on the S protein (spike protein) of SARS-CoV-2 was developed. A pilot batch of the vaccine was manufactured, and laboratory quality control of its parameters (sterility, safety, antigenic activity, and physicochemical properties) was performed.

2. Immunogenic activity of the vaccine. Various formulations of the vaccine (differing in the type and concentration of adjuvants) were administered to cats, and comparative immunogenicity studies were conducted. The results demonstrated that high titres of SARS-CoV-2-specific antibodies were detected in the serum of vaccinated cats; the antibody levels measured by ELISA were statistically significantly higher compared to the control group ($p < 0.05$); virus-neutralising antibody titres exhibited a high capacity to neutralise the reference virus strain; and

the optimal vaccine formulation was identified, which, along with high immunogenic activity, fully meets safety requirements.

3. Protective efficacy. Vaccinated cats were experimentally challenged with a virulent strain of SARS-CoV-2. The following results were obtained: the duration and magnitude of viral shedding in cats of the vaccinated group were significantly lower compared to the control group ($p < 0.01$); vaccinated animals showed no clinical signs of disease (respiratory disorders, weakness, decreased appetite) or exhibited only extremely mild symptoms; in the control group, high-level viral shedding and pronounced clinical manifestations of the disease were recorded.

4. Duration of post-vaccination immunity. The magnitude and persistence of immunity in vaccinated cats were assessed. According to the obtained data, protective antibody levels were maintained for 6 months following vaccination; high titres of virus-neutralising antibodies indicated the stability of the immune response.

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.

In all works published on the topic of the dissertation, the doctoral student's contribution is fundamental

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.