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Development of Carnivac-Cov vaccine against coronavirus infection (COVID-19) in carnivores

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SUMMARY
Development of specific protection in susceptible carnivores against COVID-19 caused by zoonotic agent is of great importance for maintaining epidemic and veterinary favourable situation in the Russian Federation and prevention of new coronavirus infection spread in humans. Development of inactivated adsorbed whole-virion vaccine (Carnivac-Cov) against coronavirus infection (COVID-19) for carnivores intended for specific disease prevention in fur animals and pet animals (cats and dogs) and tests of the vaccine for its safety and effectiveness are described in the paper. The vaccine was developed and tested at the FGBI "ARRIAH" (Vladimir) subordinated to the Rosselkhoznadzor in accordance with the Russian Federation legislation requirements. Clinical trials were carried out on fur farms, in veterinary clinics and animal shelters. More than 330 animals (fur animals, cats, dogs) were involved in the preclinical and clinical trials. The trials have demonstrated that the vaccine is safe for target animals. Carnivac-Cov vaccine administered twice intramuscularly at the dose of 1.0 cm^3 induces anti-SARS-CoV-2 immune response 14 days after the second administration that lasts for at least 6 months. The vaccine transportation, storage and application do not require any specific protective equipment. The vaccine can be used on fur farms and in veterinary clinics without limitations. Carnivac-Cov is the first tool for specific COVID-19 prevention in animals. The vaccine has successfully passed comprehensive quality control and is registered in the Russian Federation.

Keywords: SARS-CoV-2, prevention, COVID-19, vaccine safety and effectiveness, fur animal farming, pet animals.

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Создание вакцины против коронавирусной инфекции (COVID-19) плотоядных животных «Карнивак-Ков»

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РЕЗЮМЕ
Для обеспечения эпизоотического и ветеринарного благополучия Российской Федерации и предотвращения распространения новой коронавирусной инфекции среди людей первостепенное значение имеет вопрос формирования специфической защиты среди восприимчивых плотоядных животных против COVID-19, возбудитель которого обладает зоонозным потенциалом. В статье представлены результаты разработки, а также оценки безвредности и эффективности инактивированной сорбированной цельновирионной вакцины против коронавирусной инфекции (COVID-19) для плотоядных животных «Карнивак-Ков», предназначенной для специфической профилактики заболевания пушных зверей и животных-компаньонов (собак и кошек). Разработку

и контроль качества препарата осуществляли согласно требованиям законодательства Российской Федерации на базе подведомственного Россельхознадзору ФГБУ «ВНИИЗЖ» (г. Владимир). Клинические испытания проводили в условиях звероводческих хозяйств, ветеринарных госпиталей и приютов для животных. В общей сложности при проведении доклинических и клинических исследований участвовало более 330 голов животных (пушные звери, кошки, собаки). Проведенные испытания препарата показали его безвредность для целевых животных. Через 14 суток после двукратного внутримышечного введения иммунизирующей дозы (1,0 см^3) «Карнивак-Ков» вызывает формирование иммунного ответа против SARS-CoV-2 продолжительностью не менее 6 мес. Транспортировка, хранение и применение препарата не требует обеспечения особых условий. Вакцина может свободно использоваться в условиях звероводческих хозяйств и ветеринарных клиник. «Карнивак-Ков» является первым в мире инструментом специфической профилактики COVID-19 у животных. Препарат успешно прошел всесторонний контроль качества и зарегистрирован на территории Российской Федерации.

Ключевые слова: SARS-CoV-2, профилактика, COVID-19, безвредность и эффективность вакцины, пушное звероводство, животные-компаньоны.

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INTRODUCTION

The first human cases of COVID-19, the disease caused by new SARS-CoV-2 coronavirus, were officially reported in Wuhan, People's Republic of China, in December 2019. Since that time the disease has spread around the world, affected more than 170 mln people and caused more than 3.5 mln human deaths. Cases of COVID-19 transmission from human to animals, its agent circulation in animal population and its transmission back to humans were officially reported [1, 2]. COVID-19 infection agent is an enveloped single-stranded non-segmented RNA-virus, SARS-CoV-2, belonging to *Coronaviridae* family, *Betacoronavirus* genus. *Coronaviridae* family members are the major pathogens of mammals (including humans), amphibians and birds [3–5].

Coronaviruses have multiple virus subgroups. Alpha- and beta-coronaviruses (including coronavirus causing common cold in humans) as a rule affect mammals; gamma- and delta-coronaviruses generally cause infectious diseases in birds and fish. Many of the common coronaviruses causing diseases in domestic animals, such as feline enteral coronavirus, belong to alpha coronaviruses. SARS-CoV-2 affecting the human respiratory system belongs to beta-coronavirus group [4, 6–8].

The initial source of COVID-19 infection agent has not yet been identified, but the World Health Organization (WHO) and World Organization for Animal Health (OIE) are both of opinion that bats are the most probable environmental reservoir of SARS-CoV-2. However, the agent could undergo passages in an animal of other species that was an intermediate host, before it infected the human for the first time. Such intermediate host is supposed to be a livestock animal, wild animal or farmed wild animal. The role of animals in the infection spread is not clearly defined. However, according to the OIE, some animal species are susceptible to COVID-19. Zoonotic potential of the agent was confirmed for the first time by detection of SARS-CoV-2 genome in biomaterial samples collected from dogs in Hong Kong [9–13].

SARS-CoV-2-susceptibility of animals is currently not sufficiently understood, but there are some reports on experimental infection of ferrets, cats, dogs and pigs. It was demonstrated that SARS-CoV-2 effectively replicated in cats and ferrets but dogs were low susceptible [4, 11]. B. S. Pickering et al. showed that pigs were susceptible to nasal infection with SARS-CoV-2 at a high dose [12].

COVID-19 agent was identified and reported in mink population in 10 countries: Canada, Denmark, France, Greece, Italy, Lithuania, the Netherlands, Spain, Sweden and the United States of America. First two disease outbreaks on mink farms were reported in the Netherlands in April 2020. Most affected farms reported SARS-CoV-2 infection cases in their workers that supposed coronavirus transmission from humans (animal owner or handlers) to minks and was indicative of the agent ability to become a reverse zoonosis [1, 2, 13–15]. The situation was the most complicated in Denmark where more than 17 mln minks were culled on fur farms due to partially detection of SARS-CoV-2 mutation (cluster 5) variant that was able to transmit from animals to humans [1, 2].

Thereafter, cases of SARS-CoV-2 infection in animals of different species with respiratory and intestinal signs were detected in several countries.

Emerging infectious diseases often pose threat to wildlife and biological diversity. Fur animals escaping from the farm can become the virus-maintaining source and can cause agent spillover to sympatric wild animal species in case of susceptible species presence. Currently available information is not sufficient to assess the probability of SARS-CoV-2 reservoir establishment in susceptible wild animals.

Mink escaping from the farm has always been a problem for the countries where fur farming exists or has existed. It is believed that the number of escapes increases during mass culling. It was found in one region of Denmark that the majority of the minks living in the region were born on a farm and then escaped. This is indicative of the

role of the farms as an actual sources for wild population replenishment and maintaining of mink population at large size. Similar conclusions were made in other countries. Escaped minks come into contact with wild minks: hybridization between farmed and wild individuals in the wild were documented.

The first free-ranged local wild mink with confirmed SARS-CoV-2 infection was detected in Utah State (USA) in December 2020. Phylogenetic analysis of the recovered virus isolate confirmed that it was closely related to the virus isolated from the animals on a mink farm. This suggests that the wild mink has been infected through indirect or direct contact to the infected farmed minks [14, 15].

The risk of SARS-CoV-2 infection of humans by fur animals poses a serious threat to human health and socio-economic development. The agent can be transmitted from fur animals to wild animals through direct contact between wild and infected farmed animals as well as indirectly via infected carcasses, wastes and other contaminated objects, as a result of direct and indirect contacts between fur animals/contaminated objects and stray animals. Such stray animals could be an intermediate species and transmit the virus to susceptible wild animals. SARS-CoV-2 spread on mink farms creates new opportunities for its evolution due to facilitating the agent transmission to wild animal species that could become the virus reservoirs [1, 2, 13, 15].

Thus, development of specific anti-COVID-19 protection in susceptible carnivores is of utmost importance for maintaining favorable veterinary situation in the Russian Federation and prevention of the disease spread in humans.

Considering the abovementioned, the Rosselkhoznadzor-subordinated FGBI "ARRIAH" was tasked to develop safe and effective vaccine against coronavirus infection (COVID-19) for carnivores and register it in the Russian Federation.

MATERIALS AND METHODS

Carnivac-Cov vaccine was developed and tested for its quality in accordance with Federal Law No. 61-FZ on circulation of medcines and Order of the Ministry of Agriculture of the Russian Federation No. 101 on approval of the rules for preclinical testing of veterinary medicinal products, clinical trials of veterinary medicinal products, tests of veterinary medicinal products for bioequivalence.

Four pilot vaccine batches were used for preclinical and clinical tests.

Carnivac-Cov vaccine active substance is inactivated SARS-CoV virus. Aluminum hydroxide was used as an adsorbing agent. All vaccine components were thoroughly tested during incoming quality control including tests for sterility and antigen innocuity in Vero C1008 by three consecutive passages.

Preclinical tests were performed at the FGBI "ARRIAH"; 130 animals of different species (ferrets, minks, dogs, cats) were used for preclinical trials. Clinical trials were performed in 200 target animals (cats, dogs, minks, Arctic foxes, etc.) on fur farms, in veterinary clinics and animal shelters.

The animals were kept and used in accordance with the Guidelines for laboratory animal keeping and use (Directive 2010/63/EU of the European Parliament and Council of the European Union of 22 September 2012).

Serum samples from the animals were tested for specific antibodies against SARS-CoV-2 in accordance with the Methodical Guidelines for detection of anti-SARS-CoV-2 antibodies with enzyme-linked immunosorbent assay in sera of susceptible animals [16].

RESULTS AND DISCUSSION

Carnivac-Cov vaccine was examined for the following during its preclinical tests: interaction with other veterinary medicinal products, toxicity for target and laboratory animals, vaccine tolerability in healthy animals, immunity duration.

Ferrets, minks, dogs and cats were used to determine the interaction of the vaccine with other immunologicals. Animals of each species were divided into four groups isolated from each other, 5 animals per group. The animals of the first groups were immunized with Carnivac-Cov vaccine, animals of the second groups were simultaneously immunized with Carnivac-Cov vaccine and anti-rabies vaccine, animals of the third groups were vaccinated with the anti-rabies vaccine 5 days after Carnivac-Cov administration and animals of the fourth groups remained non-immunized.

Blood samples were collected from the animals 14 days after their vaccination with Carnivac-Cov for sera preparation; the sera were tested for specific antibodies against SARS-CoV-2 with enzyme-linked immunosorbent assay (ELISA). Test results showed that the antibody levels in Carnivac-Cov vaccine-immunized test animals were 1:200–1:800 [16].

No signs of depression, loss of appetite, body temperature rise or other clinical disorders were detected in test animals during observation period of 35 days that together with serological test results demonstrated Carnivac-Cov vaccine compatibility with other veterinary medicinal products.

The vaccine was tested for its toxicity in ferrets, minks, dogs, cats and white mice. Animals of each species were divided into two groups, 5 animals per group. One immunizing dose (1.0 cm^3) of the vaccine was administered intramuscularly to the animals of the first groups. The vaccine was administered three times during the day every 6 hours. The animals of the second groups remained intact (control groups). The animals of all groups were observed for clinical signs including body temperature measuring and recording the observation results during 14 days after the vaccine administration. The animals were euthanized on day 15 after the experiment had started and then subjected to post-mortem examination for possible organ and tissue lesions.

No intoxication signs were detected in animals of all test groups: general body condition, body temperature, water and feed intake, etc. were similar to that ones in control groups of animals. Obtained data showed that Carnivac-Cov vaccine was not toxic for the said animal species.

The vaccine was tested for its tolerability in healthy animals. The tests were carried out in ferrets, minks, dogs, cats injected intramuscularly by the recommended vaccine dose (1.0 cm^3) as well as by the vaccine doses that were 5, 10 and 50 times higher than the recommended dose. Each vaccine dose was administered intramuscularly twice with a 21-day interval. The animals of all groups were clinically observed for 35 days after the first vaccine administration. Afterwards, the animals were euthanized and subjected to post-mortem examination. The test findings showed that the vaccine administered at the recommended doses or at the excessive doses induced no local reactions and had

no lethal effect. Post-mortem examination showed that organs of all test animals were normal. Thus, obtained results have shown that Carnivac-Cov vaccine is safe and non-reactogenic for carnivores.

Tests for determination of the vaccine immunizing dose were carried out in 2 months-old ferrets, minks, dogs and cats (35 animals of each species). The animals were divided into isolated groups, 5 animals per group, were intramuscularly injected with the vaccine at a dose of 0.5; 1.0 and 2.0 cm^3 once and injected twice at the same doses at a 21-day interval. The animals were examined for clinical signs with daily thermometry during the whole observation period.

Blood samples were collected from all animals 14 days after the last pilot vaccine administration. The prepared sera were tested with ELISA for detection of specific anti-SARS-CoV-2 antibodies.

ELISA tests showed that average specific anti-SARS-CoV-2 antibody level in the animals vaccinated with Carnivac-Cov vaccine at a dose of 0.5 cm^3 was significantly lower than that ones in animals immunized with the vaccine at doses 1.0 and 2.0 $см^3$ (Table).

Thus, obtained results indicated that the minimal immunizing dose of the pilot vaccine was 1.0 cm^3 when the vaccine was administered intramuscularly twice at a 21-day interval.

Tests for assessment of postvaccinal immunity duration were carried out in ferrets, minks, dogs and cats (13 animals of each species) divided into groups isolated from each other. Animals of tests groups (10 animals of each species per group) were immunized with Carnivac-Cov vaccine by double intramuscular injection of 1.0 cm^3 at a 21-day interval. The animals of control groups (3 animals of each species per group) remained non-vaccinated.

Blood samples were collected from all animals 2, 4 and 6 months after immunization and the prepared sera were tested with ELISA for detection of specific anti-SARS-CoV-2 antibodies.

Sera test results showed that average specific anti-SARS-CoV-2 antibody level in samples from the immunized animals (test groups) was 1:420–1:520 during 6 months. Whereas, no specific anti-SARS-CoV-2 antibodies were detected in sera collected from non-vaccinated animals (control groups). Thus, the test results show that Carnivac-Cov vaccine induces strong immunity response against coronavirus infection (COVID-19) agent in the immunized carnivores that lasts for at least 6 months.

The vaccine contains no infectious agent or toxic substances and does not pose a potential threat to humans and environment.

Carnivac-Cov vaccine was proven safe and effective during the preclinical trials and that allowed for clinical trials of the vaccine in cats, dogs, fur animals (minks, Arctic foxes, foxes). The trials were carried out in target animals (cats, dogs, minks, Arctic foxes, foxes) of different ages on fur farms, in veterinary clinics and animal shelters. The animals were injected intramuscularly twice with Carnivac-Cov vaccine at a dose of 1.0 $см^3$ with a 21-day interval (Fig. 1–3).

No local tissue reactions to the vaccine injection, disease signs or animal deaths were detected in the animals during the observation period.

Blood samples were taken from the immunized animals (cats, dogs, fur animals) 2, 4 and 6 months after vaccination to confirm duration of the postvaccinal immunity induced by Carnivac-Cov in target animals in the field. Prepared sera were tested with ELISA for specific anti-SARS-CoV-2 antibodies.

Tests showed that average specific anti-SARS-CoV-2 antibody levels in sera collected from the immunized animals remained high throughout the whole observation period and were as follows per group: 1:485 in cats, 1:304 in dogs, 1:500 in fur animals (Fig. 4–6). Clinical trial results confirmed Carnivac-Cov vaccine safety and effectiveness.

Successful preclinical and clinical trials of Carnivac-Cov vaccine provided grounds for preparation of the

Table
Results of testing of sera from carnivores immunized with Carnivac-Cov vaccine

Таблица
Результаты исследования проб сыворотки крови плотоядных животных, иммунизированных вакциной «Карнивак-Ков»

Group No.	Animal species	Number of animals	Injected dose, volume / number of injections	Average antibody level
1	ferrets	5	0.5 cm^3/once	1:70
2		5	0.5 cm^3/twice	1:100
3		5	1.0 cm^3/once	1:140
4		5	1.0 cm^3/twice	1:440
5		5	2.0 cm^3/once	1:160
6		5	2.0 cm^3/twice	1:480
7		5	control	< 1:50
8	minks	5	0.5 cm^3/once	1:60
9		5	0.5 $см^3$/twice	1:100
10		5	1.0 cm^3/once	1:120
11		5	1.0 cm^3/twice	1:440
12		5	2.0 cm^3/once	1:120
13		5	2.0 cm^3/twice	1:340
14		5	control	< 1:50
15	dogs	5	0.5 cm^3/once	1:80
16		5	0.5 cm^3/twice	1:90
17		5	1.0 cm^3/once	1:80
18		5	1.0 cm^3/twice	1:240
19		5	2.0 cm^3/once	1:150
20		5	2.0 cm^3/twice	1:280
21		5	control	< 1:50
22	cats	5	0.5 cm^3/once	1:70
23		5	0.5 cm^3/twice	1:110
24		5	1.0 cm^3/once	1:120
25		5	1.0 cm^3/twice	1:360
26		5	2.0 cm^3/once	1:150
27		5	2.0 cm^3/twice	1:280
28		5	control	< 1:50

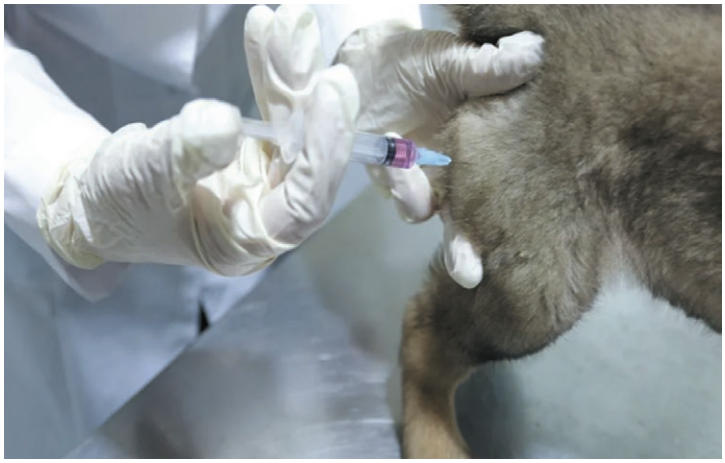

Fig. 1. Carnivac-Cov vaccine is intramuscularly administered to the puppy

Рис. 1. Внутримышечное введение вакцины «Карнивак-Ков» щенку

Fig. 2. Carnivac-Cov vaccine is intramuscularly administered to the cat

Рис. 2. Внутримышечное введение вакцины «Карнивак-Ков» кошке

Fig. 3. Carnivac-Cov vaccine is intramuscularly administered to the mink

Рис. 3. Внутримышечное введение вакцины «Карнивак-Ков» норке

registration dossier for the adsorbed inactivated vaccine against coronavirus infection (COVID-19) for carnivores and subsequent registration of the said vaccine in the Russian Federation.

CONCLUSION

Effective and safe product for specific immunization of carnivores against COVID-19 was developed based on the studies performed at the FGBI "ARRIAH". Double immunization of the animals at a dose of 1.0 cm^3 with a 21-day interval induces antibody development at the following levels: 1:485 in cats, 1:304 in dogs, 1:500 in fur animals. The vaccine has been proven safe, non-reactogenic for carnivores.

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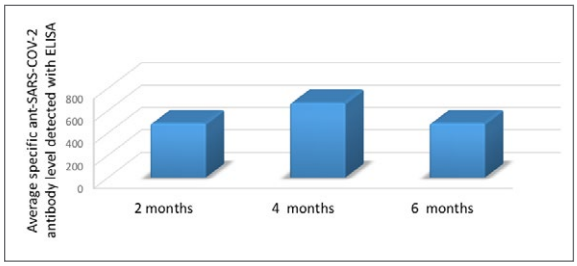

Fig. 4. Immunity duration in cats

Рис. 4. Продолжительность иммунитета у кошек

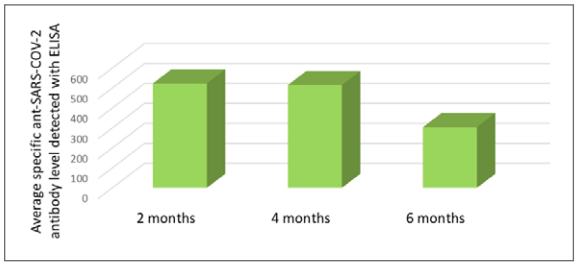

Fig. 5. Immunity duration in dogs

Рис. 5. Продолжительность иммунитета у собак

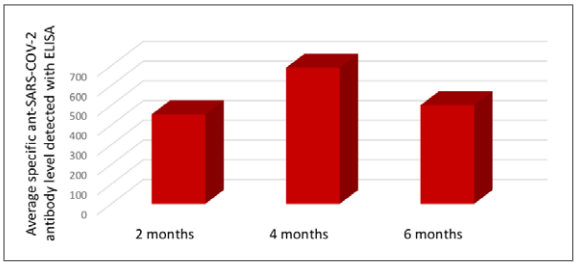

Fig. 6. Immunity duration in fur animals

Рис. 6. Продолжительность иммунитета у пушных зверей

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