REVIEWS

SARS, SARS again, and MERS. Review of animal models of human respiratory syndromes caused by coronavirus infections

Aleksey M. Nagornykh✉, Alexander I. Tyumentsev, Marina A. Tyumentseva, Vasily G. Akimkin

Central Research Institute of Epidemiology, 111123, Moscow, Russia

Since the beginning of the 21th century, major outbreaks of human respiratory syndromes caused by coronavirus infections have caused more that a million deaths on the planet. Despite the fact that the first wave of the coronavirus infection took place back in 2002, even now there is not any adequate animal model that would meet the needs of the scientific community for reproducing the pathogenesis, clinical manifestations, immunogenicity, development and testing of preventive and therapeutic compounds specific to Severe Acute Respiratory Syndrome, Middle East Respiratory Syndrome, and Coronavirus Disease 2019 (COVID-19).
The purpose of the study is to provide relevant information on known animal models of human respiratory syndromes caused by coronavirus infections and to focus the reader's attention on their adequacy, which consists in the most accurate imitation of clinical signs and pathomorphological changes.

Keywords: *coronavirus; SARS-CoV; MERS-CoV; SARS-CoV-2; animal models, review.*

Acknowledgments. The study had no sponsorship.

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

For citation: Nagornykh A.M., Tyumentsev A.I., Tyumentseva M.A., Akimkin V.G. SARS, SARS again, and MERS. Review of animal models of human respiratory syndromes caused by coronavirus infections. *Journal of microbiology, epidemiology and immunobiology = Zhurnal mikrobiologii, èpidemiologii i immunobiologii.* 2020; 97(5): 431–442.
DOI: https://doi.org/10.36233/0372-9311-2020-97-5-6

Received 16 July 2020
Accepted 28 August 2020

SARS, снова SARS и MERS. Обзор животных моделей респираторных синдромов человека, вызываемых коронавирусными инфекциями

Нагорных А.М.✉, Тюменцев А.И., Тюменцева М.А., Акимкин В.Г.

ФБУН «Центральный научно-исследовательский институт эпидемиологии» Роспотребнадзора, 111123, Москва, Россия

Крупные вспышки респираторных синдромов человека, вызываемых коронавирусными инфекциями, с начала XXI в. стали причиной гибели более миллиона человек на планете. Несмотря на то что первая волна коронавирусной инфекции случилась еще в 2002 г., до сегодняшнего дня не существует ни одной адекватной животной модели, одновременно удовлетворяющей потребности научного сообщества в воспроизведении патогенеза, клинических проявлений, иммуногенности, разработке и испытании средств специфической профилактики и терапии тяжелого острого респираторного синдрома, ближневосточного респираторного синдрома и коронавирусного заболевания 2019 г. (COVID-19).
Цель работы — представить актуальную информацию по известным животным моделям респираторных синдромов человека, вызываемых коронавирусными инфекциями, и акцентировать внимание читателя на их адекватности, заключающейся в максимально точной имитации клинических признаков и патоморфологических изменений.

Ключевые слова: *коронавирус; SARS-CoV; MERS-CoV; SARS-CoV-2; животные модели; обзор.*

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Для цитирования: Нагорных А.М., Тюменцев А.И., Тюменцева М.А., Акимкин В.Г. SARS, снова SARS и MERS. Обзор животных моделей респираторных синдромов человека, вызываемых коронавирусными инфекциями. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2020; 97(5): 431–442. DOI: https://doi.org/10.36233/0372-9311-2020-97-5-6

Поступила 16.07.2020
Принята в печать 28.08.2020

Introduction

The worldwide notoriety of coronavirus infections is due to the registration of the disease outbreak that occurred in the southern provinces of China in 2002. At that time, according to some politicians, the level of infection in the human population did not reach alarming proportions because the authorities of China poorly informed the competent divisions of the World Health Organization. In 2002–2003, the outbreak of severe acute respiratory syndrome (SARS) killed 774 people, which amounted to 9.6% of the total number of cases (8096 laboratory-confirmed diagnoses) [1]. The eating habits of the population were considered to be the cause of the outbreak: the traditional food in the southern regions of China includes meat from masked palm civets (*Paguma larvata*), raccoon dogs (*Nyctereutes procyonoides*), domestic cats (*Felis catus*), red foxes (*Vulpes vulpes*), Chinese ferret-badgers (*Melogale moschata*), and other representatives of the indigenous fauna. There are markets in Chinese settlements where these animal species are sold, both wild and breeded at special farms. The first cases of infection known as "atypical pneumonia" were reported among the personnel of these retail markets, as well as among visitors of these retail locations.

The next center of coronavirus infection with an acute respiratory syndrome was the Middle East. In 2012, the coronavirus of the Middle East Respiratory Syndrome (MERS-CoV) was isolated from a Saudi Arabian, and later cases were reported in 20 more countries around the world. In 2012–2013, more than 1900 cases of MERS-CoV infection were identified, 36% of which were lethal [2]. The exact transmission route of the virus is still not completely clear, and the pathoanatomical data of the patients who died from MERS are not available. To date, the working theory of MERS-CoV transmission is considered to be the human-camel contact. In turn, camels become infected from bats of the *Pipistrellus* and *Nycteris* genera. Although the virus transmission route from chiropterans, which are insectivorous animals, to camels also remains unclear.

For the third time, the coronavirus manifested itself again in the southern provinces of China in November 2019. The pathogen had signs similar to SARS-CoV, so it was named SARS-CoV-2. Since the first cases were reported until now, almost 40 million cases have been recorded in the world, more than 1.1 million thereof lethal[1].

Coronaviruses are widespread among representatives of the animal world. They have adapted to infect a variety of animal species, including birds, members of the feline and canine families, ungulates, mice, cetaceans, primates, ferrets, and camels. There have been described hundreds of coronaviruses that are classified into four genetically different genera: alpha and beta, affecting mainly mammals, while gamma and delta mainly infect birds [3]. Alpha, beta and delta coronaviruses can be found in domestic animals, among the etiological agents of the coronavirus infection [4].

Alphacoronaviruses affect dogs (intestinal form), cats, pigs (transmissible gastroenteritis), minks, and ferrets (intestinal and systemic forms). Betacoronaviruses initiate disease in cattle (BCoV), dogs (respiratory form), horses, and pigs (hemagglutinating encephalomyelitis), while deltacoronavirus affects pigs. It should be noted that vaccines have been developed and are actively used against a few diseases only, i.e. alphacoronaviruses of dogs and cats, as well as betacoronaviruses of cattle and pigs. These vaccines partially increase the host's resistance to *spike* glycoproteins of coronaviruses. However, the spikes themselves differ even within the same genus of coronaviruses, primarily in the receptor-binding domain, which specifically recognizes angiotensin-converting enzyme 2 (ACE2) of its host [5]. Thus, the bovine coronavirus and the pathogens of acute respiratory syndromes belong to the same genus, however BCoV belongs to Betacoronavirus lineage 2a, SARS-CoV viruses refers to Betacoronavirus lineage 2b, and MERS-CoV is the only known pathogenic human coronavirus of lineage C [6]. These lineages of coronavirus are so genetically different from each other that they stimulate the production of completely different antibodies that do not cross-react.

Despite such an apparently wide variety of animals that seem prospective as models for simulating the pathogenesis of human coronavirus infections, the number of adequate animal models is highly restricted. The problem is that the range of animals susceptible to spike glycoproteins of human coronaviruses is narrow.

[1] WHO. Novel Coronavirus (2019-nCoV). Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

DOI: https://doi.org/10.36233/0372-9311-2020-97-5-6

Animal models of SARS

The specific target for SARS-CoV is angiotensin-converting enzyme 2 [7]. Once in the host, the actively replicating virus causes damage to the lung tissue and sometimes the intestinal epithelium, manifesting itself as the interstitial pneumonia with fever and diarrhea.

Naturally, the first animal models were based on laboratory rodents, in particular mice. The data published suggest that the results of using mice as models for SARS are ambiguous. Thus, D. Wentworth and colleagues used four-week female BALB/c mice to inoculate them intranasally and orally with 2×10^5 $TCID_{50}$/ml SARS-CoV (Urbani strain). Clinical signs of the disease were manifested in the loss of up to 6% of body weight and ruffled hair in animals from the experimental group. Virus neutralizing antibodies were detected from 7th to 28th days after infection, and their maximum titer was reached on the 28th day. According to the members of the research team, serological data, as well as the detection of subgenomic RNA in the lungs and intestines of mice, evidenced that SARS-CoV replicated in these tissues. The results of this study demonstrated the early achievement of peak values of the virus concentration in the lung tissue and intestines (3–5 days after infection) and the subsequent clearance, which completed by the 10th day after the inoculation of the pathogen [8].

According to statistics, SARS has the most severe progression in the elderly, which prompted researchers to use aged wild-type mice as models. Thus, A. Roberts et al. carried out intranasal infection of 12–14-month old BALB/c mice with 1×10^5 $TCID_{50}$ SARS-CoV (Urbani strain). Clinical signs similar to SARS, with the exception of fever, which was never diagnosed, appeared 3 days after the inoculation of the virus. However, clearance began by the 7th day, and no lethal cases were reported at all [9].

The absence of a high level of specific lethality, as well as the late onset of the seroconversion peak in wild-type animals, led to the development of transgenic mice capable of expressing human ACE2 (hACE2). In 2007, two research groups independently published the results of inoculation of SARS-CoV in hACE2 transgenic mice [10, 11]. In both cases, the intranasal infection of the developed models was carried out using the Urbani strain. During the study, the infected transgenic mice showed a decrease in body weight, lower activity and shortness of breath on the 3–5th day after the inoculation of the pathogen. On the 7–8th day after the infection, all mice from the experimental groups died, and earlier death was observed in animals with the highest number of copies of the hACE2 transgene (fist deaths were reported on the 4th day) [10]. The histological examination showed infiltration of the lungs with macrophages and lymphocytes, as well as increased expression of proinflammatory cytokines and chemokines in the lungs and brain, which is similar to the SARS scenario in humans [11]. In addition, to demonstrate the moderate adequacy of the obtained models, the authors carried out a study of the effectiveness of preemptive therapy of the infected animals with human monoclonal antibodies specific to SARS-CoV. It was found that the intravenous administration of monoclonal antibodies at a dose of 25 mg/kg of body weight 1 day before the inoculation of the pathogen completely prevented death in mice [10].

Even this model is not completely adequate, although it was a big step in understanding the pathogenesis of SARS: despite a tenfold decrease in the titer of the administered virus [10], the lethality in transgenic mice was much earlier than it was observed in humans.

Another research team carried out intranasal infection with recombinant strains of SARS-CoV with deletions of the ORF7ab region in the amount of 1×10^3 $TCID_{50}$/ml of immunodeficient Syrian hamsters, in which the immunosuppression was induced by cyclophosphamide, and an increase in the dose and frequency of administration of cyclophosphamide increased the body weight loss and lethality in direct proportion. On the other hand, the cause of the increase in these indicators remained unclear, i.e. increased or prolonged viral replication or increased tissue damage caused by cytokines. At the same time, the histological assessment of the organs of infected immunodeficient hamsters revealed the existence of chronic interstitial bronchopneumonia on the 19th day after the inoculation of the virus. The changes found in the heart, kidneys and nasal cavity were sporadic, with a small degree of damage. It is doubtful that this model would be useful in vaccine research, but it may be adequate in assessing the effectiveness of antiviral compounds or therapies [12].

Domestic cats and ferrets with their own coronavirus pathogens have also been susceptible to carriage of SARS-CoV. To support this theory, cats and ferrets were intratracheally inoculated with 1×10^6 $TCID_{50}$ of isolate obtained from patient 5568, who died of SARS; the isolate was four-time passaged *in vitro* using the Vero 118 cell line. During the observation, the cats did not show clinical signs of the developing infection, while 3 ferrets developed a state of lethargy on days 2–4 after the infection, 1 of them died on the 4th day after the inoculation of the pathogen. Starting from day 2 after the infection, SARS-CoV was detected in both cats and ferrets in the examination of pharyngeal swabs using the reverse transcription polymerase chain reaction technique. Virus shedding lasted until the 10th day in cats and until the 14th day in ferrets, and the total virus shedding was reported until the 8th day only. As for the examination of nasal and rectal swabs, only 2 cats were confirmed to have virus shedding on the 4th and 6th days after the infection. The quantification in lung homogenates demonstrated lower viral titers in the cats ($1 \times 10^3 \pm 0.51$ $TCID_{50}$/ml), while in the ferrets titers

were higher ($1 \times 10^6 \pm 0.70$ $TCID_{50}$/ml). The titers of neutralizing antibodies in all the animals reached the level of 40–320 by the 28th day. At the same time, the cats from the control group, not infected with SARS-COV, kept together with the animals from the experimental group, showed a lower level of seroconversion (titers of neutralizing antibodies were 40 and 160) by the same time without clinical signs of the disease. The uninfected ferrets developed torpidity and conjunctivitis and died on the 16th and 21st days, while there was no confirmation that the animals died from SARS-CoV-associated pneumonia, despite the posthumous virus isolation from lung samples in one of the dead ferrets [13]. Certainly, the results of this experiment were useful for understanding the possibilities of virus transmission from animals to humans, however, the rapid clearance of the virus and the absence of evident clinical signs characteristic of a patient with SARS did not allow these models to become adequate for the evaluation of therapy protocols.

The synthesized monoclonal antibody IgG1 CR3014 was tested on ferrets infected with the HKU-39849 strain that can be hosted by humans, ferrets, and rhesus monkeys. Administration of monoclonal antibodies 1 day prior to the infection significantly reduced the viral replication in the lung tissues, and the SARS-CoV isolation with pharyngeal secretions was completely excluded in 75% of the treated animals. However, the remaining 25% of the animals were shedding the virus at the same rate as the untreated ferrets [14].

As for the experimental infection of the animals that are physiologically and anatomically the closest ones to humans and accessible in laboratory — non-human primates (NHP), the largest amount of data was obtained from studies on rhesus monkeys (*Macaca mulatta*), African green monkeys (*Chlorocebus sabaeus*) and crab-eating macaques (*Macaca fascicularis*). The extensive study [15] used all three of the aforementioned primate species. The infection was performed in two ways — intranasally and intratracheally with the Urbani strain, 1 ml 1×10^6 $TCID_{50}$/ml. Monitoring of the condition of animals showed that the virus was able to replicate in the lung tissue of NHP, while the level of serum neutralizing antibodies correlated in direct proportion with the level of viral replication in the respiratory airway. None of the monkeys was reported to show signs of a febrile respiratory illness. It is of interest that the highest level of viral replication in the upper and lower respiratory airways was in the African green monkeys (mean value 10^3 $TCID_{50}$/ml), and the lowest one was in the rhesus monkeys. The mean titer values of neutralizing antibodies in the rhesus monkeys were 1:27, in the crab-eating macaques — 1: 31, and in the African green monkeys — 1: 57 [15].

Another research team limited their model selection to crab-eating macaques. The animals were infected with the Urbani strain, however, there were selected several methods of inoculation of the pathogen in the organism of the models. The animals of the first group were infected intranasally and intrabronchially, the second group — intranasally and conjunctivally, the third group was administered an intravenous injection of the virus. As noted, the animals of the first two groups had clinical signs of mild to moderate disease, they showed the production of antibodies and radiographically confirmed pneumonia, however, like in the previous study, the primates did not have the main symptom of SARS, i.e. fever. The virus titer by day 28 after the infection was zero in the vast majority of crab-eating macaques [16]. Additional studies showed that the African green monkeys and crab-eating macaques could not be re-infected with SARS-CoV, at least in the short term.

Considering the aforesaid, the usefulness of NHP models is certainly high, but the demonstrated clinical signs impose restrictions on the use of primates for studying the pathogenic or immunogenic properties of SARS-CoV.

Animal models of MERS

It is absolutely clear that infecting naturally susceptible animals such as camels with the MERS virus strain is a very expensive study, especially since camels will not be able to reflect the entire pathogenesis of the infection. In addition, experimental infection of animal respiratory airway cell cultures, which is used to simulate human respiratory diseases (wild-type mice, hamster, ferret) in order to identify prospective candidates for the role of animal model, showed the inability of MERS-CoV to replicate in these cells [17]. This is caused by differences in amino acid composition in the extracellular domain of dipeptidyl peptidase 4 (DPP4), which is a specific receptor for the MERS-CoV S-glycoprotein. The phylogenetic analysis of the DPP4 virus-binding domain allowed grouping human DPP4 (hDPP4), DPP4 of macaque, horse, and rabbit with DPP4 of cattle, pig, and bat, despite their great difference according to the results of the phylogenetic analysis of the complete DPP4 [18]. Therefore, attempts to reproduce MERS in immunodeficient mice strains were also unsuccessful [19].

On the other hand, the preliminary, 5 days before infection with MERS-CoV, transduction to young and elderly C57BL/6 and BALB/c mice of an adenoviral vector carrying human DPP4 (Ad5-hDPP4) allowed reaching a viral replication level of 7×10^7 plaque-forming units (PFU) per 1 g in the lung tissue by the 2nd or 3rd day after infection. At the same time, by the 7th day after the inoculation of the virus, the interstitial pneumonia was reported in animals, within 10 days after the infection, the young BALB/c mice were not gaining weight and the elderly animals of both strains were losing weight, but no deaths were reported. The young mice reached the virus clearance by the 6th to 8th days

after the infection, and the elderly mice — by the 10th to 14th days [20].

Accordingly, further research of MERS-CoV could continue in three main areas: the search for suitable animal species, the modification of traditional animals, and the adaptation of the virus to resistant animals.

Cell culture studies showed that the insertion of two amino acids corresponding to 288 and 330 in the human sequence of the mDPP4 receptor supports the attachment, entry and replication of MERS-CoV [21]. Therefore, in one series of experiments, the genome of wild-type C57BL/6J mice was edited using the CRISPR/Cas9 technology, and the pathogen was passaged 15 times in mice to impart pathogenic properties. The feature of this model is the absence of neurological signs [22]. In another study, the adaptation of the virus strain was carried out through 30 consecutive passages in mice carrying a *DPP4* gene locus replaced by the human one. As a result, the obtained virus was different from the original EMC-2012 at 3 loci, one of them being characteristic of the virion spike (T1015N) [2].

On the one hand, the indisputable usefulness of these studies consists in the development of a lethal mouse model adapted for the MERS-CoV infection, but on the other hand, the virus strain was changed. As a result, it gave a genetically modified mouse that reaches 80% lethality when infected with a genetically modified virus. Even ignoring Koch's postulates, there are many critics of this approach to the study of the pathogenesis of the disease that is deadly for some categories of people. However, any data that help understand the mechanism of the disease will be useful for the study of infections so much threatening the health of the population.

In 2017, the results of a successful development of the lethal mouse model of MERS by inoculation of a transgene containing hDPP4 cDNA into zygotes of B6C3F1/J× C57BL/6J or C57BL/6J mice were published. After the infection with MERS-CoV, the resulting Tg^+-mice developed progressive pneumonia characterized by extensive inflammatory infiltration, while brain lesions were minor [23]. The resulting transgenic mice had a dose-dependent lethal outcome with 100% mortality: after the intranasal administration of the EMC-2012 MERS-CoV strain at a dose of 1×10^6 $TCID_{50}$, the mice died within 4–6 days after the inoculation, while after the administration of a dose of 1×10^2 $TCID_{50}$ the death of 100% of animals occurred within 6–12 days [24].

Currently, the hDPP4 transgenic mouse is the only available lethal small animal model of severe MERS-CoV infection. Although these mice express hDPP4 globally in all cell types, in contrast to the normal DPP4 expression in humans, however, this model can be used to screen the effectiveness of antiviral drugs and vaccines for mitigation or prevention of the MERS-CoV-induced respiratory disease.

Taking into account that ferrets demonstrated a relative susceptibility to SARS-CoV, as well as to some other respiratory infections [25, 26], some attempts were made to reproduce the pathogenesis of MERS-CoV on these animals. However, the intranasal and intratracheal infection of ferrets with MERS-CoV at a dose of 1×10^6 $TCID_{50}$ did not induce seroconversion and the infective virus itself was not detected in the animals [18].

Since the virus binding domain of rabbit DPP4 have much in common with the similar domain in humans [18], the possibility of MERS-CoV infection of rabbits was investigated. The resulting model is of limited value, because the rabbits shed the virus from the upper respiratory airway, but they did not show symptoms of infection, so it was impossible to study the disease in the context of the development and increase of its clinical signs. The virus was typically detected in nasal swabs within 7 days after the infection [6].

Although the above models were undoubtedly a huge contribution to the study of the pathogenesis of MERS-CoV and provided the basis for screening studies of antiviral therapeutic drugs and effectiveness of vaccines, it should be recalled that rhesus monkeys were the first animal model that fulfilled Koch's postulates for MERS-CoV. 6 to 12 year old animals were infected with the virus at a dose of 7×10^6 $TCID_{50}$ in a combined manner using intranasal, intratracheal, conjunctival, and oral inoculation of the pathogen [27, 28]. All the animals developed clinical signs, such as decreased appetite, fever, rapid breathing, cough, and hunched posture, within 24 hours. The signs lasted for 4 days. Severe lesions, such as dense, edematous, light or dark red foci, developed in the lungs only. The infective virus was also isolated from the lungs and MERS-CoV RNA was found in some tissues of the upper and lower respiratory airways. MERS-CoV RNA was also identified in nasal swabs, bronchoalveolar lavage samples, and several oropharyngeal swabs. Despite the presence of the viral RNA and evidence of virus shedding from the upper respiratory airway, lesions and viral replication were observed only in the tissues of the lower respiratory airway, and the viral replication took place in pneumocytes of types I and II. The immunohistochemical assay showed that the viral antigen in the lungs was present only in the areas of pneumonia. No viral RNA was detected in the blood, as in any organs of the abdominal cavity [6].

Also, a Chinese research team showed that the production of specific antibodies against MERS-CoV in rhesus monkeys began on the 7th day after the infection and the antibody titer increased over time. In addition, the produced virus-neutralizing antibodies provided protection by preventing the development of the infection upon repeated inoculation of rhesus monkeys [29].

Based on the absence of differences between the 14 amino acid residues of human DPP4 and that of the common marmoset (*Callithrix jacchus*) in the interac-

tion regions of the receptor-binding domain of the spike glycoprotein, D. Falzarano *et al.* suggested the possibility of binding of the S-glycoprotein MERS-CoV to DPP4 of the common marmoset. Starting from the 1st day after the inoculation of the EMC-2012 strain using a combined method similar to that described earlier for rhesus monkeys, the animals showed the onset and progression of clinical signs of infection: rapid and labored breathing, decreased appetite and activity. The peak values of these clinical indicators were observed between the 4th and 6th days after the infection, and on the 13th day, the indicators returned to the baseline. Starting from the 3rd day after the infection, the animals showed a decrease in body temperature, which returned to normal by the 9th day after the inoculation with the pathogen. No clinically significant changes in the chemical composition and cytological parameters of blood were observed in any of the animals, in comparison with the rhesus monkey model [30].

Unlike the rhesus monkeys, the main sites of viral replication were pneumocytes of type I and alveolar macrophages, and the immunohistochemistry showed that these cell types were expressing DPP4. High levels of viral RNA were found in the lungs, while lower levels of RNA were present in the tissues of the upper respiratory airway and swabs taken from the nasal cavity and oropharynx, as well as in blood and some internal organs, including kidneys. The infective virus was isolated from the tissues of both upper and lower respiratory airways. The detection of viremia and viral RNA in the systems of several organs showed that MERS-CoV was widely distributed throughout the body of the marmosets, however, lesions were present in the respiratory airway only [6].

Animal models of COVID-19

At the time of the SARS-CoV-2 infection spread, the research community already had an idea of possible animal models for researching this new disease. Although SARS-CoV outbreaks occurred more than 15 years ago, research continued after their end. Therefore, models that are effective in the study of SARS-CoV were used first based on the 79–82% identity of the nucleotide sequences of SARS-CoV-2 and SARS-CoV [31, 32], and taking into account the dynamics of the increase in the number of cases and deaths caused by COVID-19, even reputable journals took the risk of publishing unreviewed reports.

One of these reports referred to the intranasal infection of hACE2-transgenic mice with the HB-01 strain of SARS-CoV-2 at a dose of 10^5 $TCID_{50}$/mouse. 5 days after the infection, these mice showed ruffled hair and 8% weight loss, with no other signs of the developing infection observed. The viral RNA was detected in the lungs and intestines 1 day after the infection. The histological examination also showed foci of interstitial pneumonia in the infected animals, while no damage was found in other organs, including the brain [33]. Similar to the vast majority of studies cited above in this review, this model is not lethal. Moreover, starting from the 7th day after the infection, the foci of pneumonia began to arrange, which places this model in the category of those suitable for studying the pathogenesis of SARS-CoV-2, but leaves many questions about its suitability for studying post-vaccination immunity.

Susceptibility testing by combined inoculation (intranasal and intravenous) of the USA-WA1/2020 SARS-CoV-2 strain at a dose of 10^5 focus-forming units to immunocompromised strains of laboratory mice (BALB/c, DBA/2J, Stat1$^{-/-}$ C57BL/6, AG129, Rag1$^{-/-}$ C57BL/6) showed no weight loss in all animals during the 1st week of the study, and the amount of viral RNA in the lungs collected 10 days after the infection was very low [34].

In continuation of this study, the same dose of the USA-WA1/2020 SARS-CoV-2 strain was inoculated to BALB/c mice by intranasal and intravenous ways 5 days after the introduction of the hACE2-encoding adenovirus (AdV-hACE2). During the 1st week, the animals lost 10–25% of their body weight. On the 4th day after the infection, high levels of SARS-CoV-2 and viral RNA were found in the lung tissue, while lower levels were present in the heart, spleen, and brain and were virtually absent in the tissues of kidneys, gastrointestinal tract and blood serum. Since the transduction of AdV-hACE2 initiates only transient sensitization of hACE2 in mice, 1000-fold decrease in the viral RNA levels occurred by 8th–10th days after the infection, although they were still easily detected. Of course, the disadvantage of this model is the use of the adenoviral vector, because it can act as an independent initiator or a protagonist of lung damage [34].

Another way to deliver hACE2 to the respiratory airway of C57BL/6J (B6J) mice is through an adeno-associated virus. The inoculation was performed intranasally with a dose of 10^6 PFU/mouse of the USA-WA1/2020 strain. No weight loss or death was reported in the mice for 14 days. The authors state that the infection process remained productive in the infected mice, but the illustrations presented demonstrate a decrease in the amount of viral RNA starting from the 2nd day after the inoculation of the pathogen and with virtually the same level of progression as in animals not infected with SARS-CoV- 2. Histopathological changes in the lungs were characterized by the presence of mild diffuse peribronchial infiltrates [35].

At present, the culmination in the development of humanized mouse models of human coronavirus diseases expressing hACE2 is the use of genome editing technology applying CRISPR/Cas9. Using this technology, the hACE2-coding cDNA sequence was integrated into exon 2, which is the first coding exon of the mouse ACE2 gene (*mACE2*). Thus, it modified the gene

DOI: https://doi.org/10.36233/0372-9311-2020-97-5-6

and stopped its expression. A guide RNA, Cas9-encoding mRNA, and donor sequence encoding hACE2 were injected into the zygotes of C57BL/6 mice. Successful insertion was confirmed in almost 22% of the resulting progeny. Further, the founders were backcrossed with C57BL/6 mice and the resulting $F1^+$ progeny were screened. Additional studies showed the absence of random insertions in all the resulting mice, while the *mACE2* gene was completely absent in homozygous individuals, but the *hACE2* gene was consistently expressed in sufficient quantities in the lungs, small intestine, spleen, and kidneys. The resulting mice were named hACE2-KI/NIFDC (hACE2-mice) [31].

To confirm the susceptibility of humanized mice, they were intranasally infected with SARS-CoV-2 at a dose of 4×10^5 PFU. It should be noted that not only young animals at the age of 4.5 weeks were infected, but also elderly mice at the age of 30 weeks, which is very important, because it is elderly people and people with chronic diseases that are at risk. Starting from the 3rd day after the inoculation of the pathogen, a decrease of up to 10% of body weight was recorded in elderly mice, but after that they recovered and no evident clinical signs of SARS-CoV-2 progression were observed in any of the animals. A sustained viral RNA replication was found in the lungs, trachea and brain tissues of hACE2-mice, regardless of their age, while the viral RNA was not detected in the spleen, kidney, liver, blood serum, and intestines, despite the fact that the feces of elderly mice contained a high level of viral RNA (2.9×10^5 copies/g). The detection of RNA in feces was consistent with data on patients who had gastrointestinal symptoms after infection with SARS-CoV-2 [36, 37]. The oral administration of the virus to hACE2-mice also led to viral replication in the trachea and lungs in 40% of animals in the amount comparable to that in animals infected by the intranasal route. Although clinical signs of the developing disease in animals were still not observed [31].

The histological examination showed the presence of age-independent interstitial pneumonia characterized by infiltration of inflammatory cells, thickening of the alveolar septum, and typical damage to the vascular system. In addition, the elderly mice showed extensive lesions of alveolar epithelial cells and focal hemorrhages, as well as increased tissue infiltration with neutrophils and macrophages, and the direct infection of macrophages in the lungs led to significant apoptosis [31], which reproduces the clinical signs in most patients affected by COVID-19. Unfortunately, the authors of the publication do not specify whether the developed model was lethal, because the animals were removed from the experiment on the 6th day after the infection. In this regard, we can talk about the unconditional value of the resulting model, but its adequacy is in doubt, and, perhaps, we will see a refutation of these doubts soon.

Based on the similarity to SARS-CoV, golden Syrian hamsters are used as models for COVID-19. Most studies on them are carried out to demonstrate the pathogenesis and possible transmission routes from an infected animal to naive hamsters [38–40]. Both intranasal administration of the virus [41] and its combination with the conjunctival route [40] were effective, and the combined route was recognized as more effective. All published results note quite a conditional manifestation of clinical signs of COVID-19, the disease often progresses with a slight loss of body weight by 10–15%, mainly in elderly animals. Gradual recovery of the body weight goes on within 7 days. The infectious viral load in the upper respiratory airway reached its peak on days 2–3 after the infection, after which it decreased rapidly [38], and the viral clearance was achieved by the 7th day. The viral antigen of the COVID-19 pathogen was detected in the epithelial cells of the duodenum on the 2nd day after the infection in the absence of signs of inflammation. In addition, viral RNA was detected in fresh fecal samples collected from days 2 to 7 after the inoculation of the pathogen [38].

The histological examination showed the presence of inflammatory infiltrates in the lung tissue of infected animals characteristic of the mild course of COVID-19 [39].

Although the initially infected hamsters were shedding the viral RNA from the nasal cavity for 10–14 days, they were able to infect healthy animals by contact and aerosol routes only during the first 3 days after the inoculation of the pathogen [38].

The study of the dose-dependent effect demonstrated the occurrence of extensive lesions to the lower respiratory airway at earlier stages in animals receiving an increased amount of viral particles, although the sizes of the lesions were the same as in the animals receiving a moderate dose by the 6th day after the infection [41].

The re-infection of hamsters with SARS-CoV-2, at least in the short term, was not possible due to the formation of virus-neutralizing antibodies [42]. They were isolated from convalescent hamsters 14 days after the infection, and their average titer was at least 1:427. The use of the sera obtained in this way significantly reduced the viral load on the lungs of the infected hamsters, but could not prevent the development of the pathology in them [39].

To date, few reports on simulating COVID-19 in ferrets have been published [43, 44]. The model developed by Y.I. Kim [44] demonstrated greater adequacy, although it is not lethal either. It differs from the previous animal models of COVID-19 by the manifestation of fever with intermittent cough, sustained weight loss and decreased activity for 4 to 6 days after the intranasal inoculation of the pathogen. Ferrets infected with SARS-CoV-2 were shedding the virus with nasal excretions, saliva, urine, and feces during 8 days after the

inoculation of the pathogen, and the greatest replication was achieved in the turbinates, trachea, lungs, kidneys, and intestines [44].

Moreover, the model could show the possibility of infection of naive ferrets by direct contact with the animals demonstrating clinical signs of the disease, although the infected naive ferrets showed only increased body temperature and reduced activity without loss of body weight. The naive animals that had indirect contact with the infected ferrets did not develop clinical signs, but the viral RNA was detected in several animals, which indicates the possibility of airborn transmission.

The developed model has already demonstrated its practical significance. It was used to test the antiviral effectiveness of drugs approved by the U.S. Food and Drug Administration (FDA) against COVID-19: lopinavir/ritonavir, hydroxychloroquine and emtricitabine/tenofovir [45].

A comparative analysis of the ACE2 receptor variations in different primate species showed that all the Old World monkeys (Catarrhini) are more likely to be highly susceptible to SARS-CoV-2, in contrast to the New World monkeys (*Platyrrhini*). This feature consists in 3 differences in amino acid residues, 2 of which — H41Y and E42Q — are the most significant [46].

The choice of NHP to simulate the COVID-19 infection is usually limited to standard NHP species: crab-eating macaques, rhesus monkeys of the Old World, and common marmosets as a New World endemic. Although there are reports of SARS-CoV-2 studies on baboons, including *Papio cynocephalus* [47].

Gender and age did not affect the development and clinical manifestations of COVID-19 in NHPs [48, 49], which confirms the hypothesis that not the old age, but the presence of concomitant diseases is the cause of high mortality among the elderly people. After the infection, the Catarrhini showed an increased body temperature, for example, it rose to 40.9°C in rhesus monkeys, while in one third of crab-eating macaques and marmosets the body temperature rose insignificantly. The disease is accompanied by a decrease in body weight: in rhesus monkeys by 6–29%, and in crab-eating macaques by 2–11% [49], while the re-infection of rhesus monkeys did not cause a relapse of COVID-19 [50]. Starting from the 10th day after the inoculation of the virus, the X-ray studies show a pulmonary pathology in Catarrhini. In the swabs, a high level of viral RNA is observed already on the 2nd day after the inoculation of the virus, and it reaches its peak on the 6th–8th days after the infection and can be detected until the 14th day after the inoculation. Compared to the anal and nasal swabs, less viral RNA is found in the laryngeal swabs,

Table 1. Characteristics of animal models of human respiratory syndromes caused by coronavirus infections from the point of view of their adequacy and capability to simulate clinical features

Animal		Observed adequate clinical features		
		SARS	MERS	COVID-19
Mice	BALB/c, young	Weight loss [8]	Respiratory distress symptoms [20]	Weight loss [34]
	BALB/c, aged	Weight loss [9]	Respiratory distress symptoms [20]	–
	C57BL/6	–	Respiratory distress symptoms [21], lethal outcome [2, 22]	–
	Transgenic	Lethal outcome [10]	Weight loss [23], lethal outcome [24]	Weight loss [33]
Golden Syrian hamsters		Lethal outcome [12]	–	Weight loss [38], respiratory distress symptoms [39, 41]
Rabbits		–	–	–
Ferrets		Lethal outcome [13]	–	Respiratory distress symptoms [44]
Cats		–	–	–
Non-human primates	Rhesus monkeys	–	Ruffled hair [28], weight loss [27–29]	Respiratory distress symptoms [47, 49]
	African Green monkeys	–	–	–
	Cynomolgus	Respiratory distress symptoms [16]	–	Respiratory distress symptoms [47, 49]
	Common marmosets	–	Ruffled hair, weight loss, respiratory distress symptoms [30]	–

although not all the Old World primates shed the virus in feces. The viral RNA appears in the peripheral blood on the 2nd–6th days after the infection and disappears by the 10th day still remaining in the spleen [49]. Unlike the Catarrhini, low levels of viral RNA are detected in the swabs of the New World primates during 2 weeks after the infection.

Histopathological changes in the Catarrhini range from extensive pulmonary hemorrhages [49] to multifocal interstitial pneumonia [47]. Edema of the bronchopulmonary and mediastinal lymph nodes, exudative pericarditis and inflammation of the mesenteric lymph nodes complete the pathological scenario. Unlike the Old World primates, the Platyrrhini showed a slight infiltration of the destroyed alveolar septa with inflammatory cells. Minor hemorrhages are present in the spleen parenchyma, the germinal center of which was in a state of active proliferation [49].

Despite a relatively successful simulation of the COVID-19 infection by the macaques, which constituted the basis for the study of the expected antiviral effectiveness of, for example, hydroxychloroquine [51], it is believed that baboons are a better model because the pathology that develops in them is more extensive and accompanied by more widespread and severe inflammatory lesions as compared with rhesus monkeys [47]. At the same time, the use of the New World primates, for example, common marmosets, is at least irrational, because the number of allowed control procedures is limited, clinical signs of the disease are not pronounced, and viral RNA is not detected in tissue samples obtained from the necropsy [49].

Table 2. Characteristics of animal models of human respiratory syndromes caused by coronavirus infections from the point of view of their adequacy and capability to simulate pathomorphological changes

Animal		Detected adequate pathomorphological changes		
		SARS	MERS	COVID-19
Mice	BALB/c, young	–	Damage to the organs of the lower respiratory system [20]	Damage to the organs of the lower respiratory system [34]
	BALB/c, aged	Damage to the organs of the upper and lower respiratory system [9]	Damage to the organs of the lower respiratory system [20]	–
	C57BL/6	–	Damage to the organs of the lower respiratory system [2, 20, 22]	Damage to the organs of the lower respiratory system [35]
	Transgenic	Damage to the organs of the lower respiratory system, gastrointestinal tract and central nervous system [10]	Damage to the organs of the lower respiratory system [23, 24], gastrointestinal tract and central nervous system [24]	Damage to the organs of the lower respiratory system [31, 33]
Golden Syrian hamsters		Damage to the organs of the upper and lower respiratory system [12]	–	Damage to the organs of the lower respiratory system [38, 39, 41] and gastrointestinal tract [39]
Rabbits		–	Damage to the organs of the lower respiratory system [6]	–
Ferrets		Damage to the organs of the upper and lower respiratory system [13]	–	Damage to the organs of the lower respiratory system [44]
Cats		Damage to the organs of the upper and lower respiratory system [13]	–	Damage to the organs of the upper and lower respiratory system [43]
Non-human primates	Rhesus monkeys	Damage to the organs of the lower respiratory system [15]	Damage to the organs of the lower respiratory system [27–29]	Damage to the organs of the lower respiratory system [47, 49]
	African Green monkeys	Damage to the organs of the lower respiratory system [15]	–	–
	Cynomolgus	Damage to the organs of the lower respiratory system [15]	–	Damage to the organs of the lower respiratory system [47, 49]
	Common marmosets	–	Damage to the organs of the lower respiratory system [30]	–

Conclusion

As for the vast majority of human diseases, there is no unambiguous adequate animal model for coronavirus infections accompanied by acute respiratory syndrome. This article is an attempt to clarify the animal models that adequately reproduce clinical (Table 1), pathological (Table 2) and other signs of human respiratory syndromes caused by coronavirus infections.

Perhaps the study of the natural reservoirs of pathogens would shed light on the etiology of the emergence of such mutations in them that are so dangerous for humans. Unfortunately, the animals, which are main suspected sources of infection, have a hidden way of life and are insufficiently studied. In addition, it is quite difficult to provide them with appropriate care in the conditions of research vivaria for their humanitarian study. Therefore, we can only use models based on standard types of laboratory animals so far, studying pathogenesis and symptoms on one spices, and specific therapy and immunoprophylaxis on others. Thus, the model of transgenic mice expressing hACE2 proposed by P.B. McCray [10] proved to be the most adequate among the SARS small laboratory animal models, because it demonstrates clinical signs and lethality similar to humans. The hDRR4-transgenic mice possessed similar qualities in the MERS study [24], although the rhesus monkeys also showed a clinical scenario similar to human patients [27, 28], and their virus neutralizing antibodies provided protective immunity that prevented the development of infection upon re-infection [29]. As for COVID-19, it is possible to distinguish NHP models, especially rhesus monkeys and baboons [47], which demonstrated a high level of similarity of clinical manifestations and pathological scenario to human patients.

ЛИТЕРАТУРА/REFERENCES

1. Cherry J.D. The chronology of the 2002–2003 SARS mini pandemic. *Paediatr. Respir. Rev*. 2004; 5(4): 262–9. https://doi.org/10.1016/j.prrv.2004.07.009
2. Li K., Wohlford-Lenane C.L., Channappanavar R., Park J.E., Earnest J.T., Bair T.B., et al. Mouse-adapted MERS coronavirus causes lethal lung disease in human DPP4 knockin mice. *Proc. Natl. Acad. Sci. USA*. 2017; 114(15): E3119–28. https://doi.org/10.1073/pnas.1619109114
3. Ye Z.W., Yuan S., Yuen K.S., Fung S.Y., Chan C.P., Jin D.Y. Zoonotic origins of human coronaviruses. *Int. J. Biol. Sci*. 2020; 16(10): 1686–97. https://doi.org/10.7150/ijbs.45472
4. Decaro N., Martella V., Saif L.J., Buonavoglia C. COVID-19 from veterinary medicine and one health perspectives: What animal coronaviruses have taught us. *Res. Vet. Sci*. 2020; 131: 21–3. https://doi.org/10.1016/j.rvsc.2020.04.009
5. Wan Y., Shang J., Graham R., Baric R.S., Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J. Virology*. 2020; 94(7): e00127-20. https://doi.org/10.1128/jvi.00127-20
6. Baseler L., de Wit E., Feldmann H. A comparative review of animal models of Middle East respiratory syndrome coronavirus infection. *Vet. Pathol*. 2016; 53(3): 521–31. https://doi.org/10.1177/0300985815620845
7. Li W., Zhang C., Sui J., Kuhn J.H., Moore M.J., Luo S., et al. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. *EMBO J*. 2005; 24(8): 1634–43. https://doi.org/10.1038/sj.emboj.7600640
8. Wentworth D.E., Gillim-Ross L., Espina N., Bernard K.A. Mice susceptible to SARS coronavirus. *Emerg. Infect. Dis*. 2004; 10(7): 1293–6. https://doi.org/10.3201/eid1007.031119
9. Roberts A., Paddock C., Vogel L., Butler E., Zaki S., Subbarao K. Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans. *J. Virol*. 2005; 79(9): 5833–8. https://doi.org/10.1128/JVI.79.9.5833-5838.2005
10. McCray P., Pewe L., Wohlford-Lenane C., Hickey M., Manzel L., Shi L., et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *J. Virol*. 2006; 81(2): 813–21. https://doi.org/10.1128/JVI.02012-06
11. Tseng C., Huang C., Newman P., Wang N., Narayanan K., Watts D., et al. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human angiotensin-converting enzyme 2 virus receptor. *J. Virol*. 2006; 81(3): 1162–73. https://doi.org/10.1128/JVI.01702-06
12. Schaecher S.R., Stabenow J., Oberle C., Schriewer J., Buller R.M., Sagartz J.E., et al. An immunosuppressed Syrian golden hamster model for SARS-CoV infection. *Virology*. 2008; 380(2): 312–21. https://doi.org/10.1016/j.virol.2008.07.026
13. Martina B.E., Haagmans B.L., Kuiken T., Fouchier R.A., Rimmelzwaan G.F., van Amerongen G., et al. SARS virus infection of cats and ferrets. *Nature*. 2003; 425(6961): 915. https://doi.org/10.1038/425915a
14. ter Meulen J., Bakker A.B., van den Brink E.N., Weverling G.J., Martina B.E., Haagmans B.L., et al. Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. *Lancet*. 2004; 363(9427): 2139–41. https://doi.org/10.1016/S0140-6736(04)16506-9
15. McAuliffe J., Vogel L., Roberts A., Fahle G., Fischer S., Shieh W.J., et al. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology*. 2004; 330(1): 8–15. https://doi.org/10.1016/j.virol.2004.09.030
16. Lawler JV, Endy T.P., Hensley L.E., Garrison A., Fritz E.A., Lesar M., et al. Cynomolgus macaque as an animal model for severe acute respiratory syndrome. *PLoS Med*. 2006; 3(5): e149. https://doi.org/10.1371/journal.pmed.0030149
17. van Doremalen N., Miazgowicz K., Milne-Price S., Bushmaker T., Robertson S., Scott D., et al. Host species restriction of middle east respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase 4. *J. Virol*. 2014; 88(16): 9220–32. https://doi.org/10.1128/JVI.00676-14
18. Raj V.S., Smits S.L., Provacia L.B., van den Brand J.M., Wiersma L., Ouwendijk W.J., et al. Adenosine deaminase acts as a natural antagonist for dipeptidyl peptidase 4-mediated entry of the middle east respiratory syndrome coronavirus. *J. Virol*. 2013; 88(3): 1834–8. https://doi.org/10.1128/JVI.02935-13
19. Coleman C.M., Matthews K.L., Goicochea L., Frieman M.B. Wild-type and innate immune-deficient mice are not susceptible to the Middle East respiratory syndrome coronavirus. *J. Gen. Virol*. 2014; 95(Pt. 2): 408–12. https://doi.org/10.1099/vir.0.060640-0
20. Zhao J., Li K., Wohlford-Lenane C., Agnihothram S.S., Fett C., Zhao J., et al. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proc. Natl. Acad. Sci. USA*. 2014; 111(13): 4970–5. https://doi.org/10.1073/pnas.1323279111
21. Cockrell A.S., Peck K.M., Yount B.L., Agnihothram S.S., Scobey T., Curnes N.R., et al. Mouse dipeptidyl peptidase 4 is not a functional receptor for Middle East respiratory syndrome coronavirus infection. *J. Virol*. 2014; 88(9): 5195–9. https://doi.org/10.1128/JVI.03764-13
22. Cockrell A.S., Yount B., Scobey T., Jensen K., Douglas M., Beall A., et al. A mouse model for MERS coronavirus-induced

acute respiratory distress syndrome. *Nat. Microbiol*. 2016; 2(2): 16226. https://doi.org/10.1038/nmicrobiol.2016.226
23. Agrawal A.S., Garron T., Tao X., Peng B.H., Wakamiya M., Chan T.S., et al. Generation of a transgenic mouse model of Middle East respiratory syndrome coronavirus infection and disease. *J. Virol.* 2015; 89(7): 3659–70. https://doi.org/10.1128/JVI.03427-14
24. Tao X., Garron T., Agrawal A.S., Algaissi A., Peng B.H., Wakamiya M., et al. Characterization and demonstration of the value of a lethal mouse model of Middle East respiratory syndrome coronavirus infection and disease. *J. Virol.* 2015; 90(1): 57–67. https://doi.org/10.1128/JVI.02009-15
25. Enkirch T., von Messling V. Ferret models of viral pathogenesis. *Virology*. 2015; 479-480: 259–70. https://doi.org/10.1016/j.virol.2015.03.017
26. Wong J., Layton D., Wheatley A.K., Kent S.J. Improving immunological insights into the ferret model of human viral infectious disease. *Influenza Other Respir. Viruses*. 2019; 13(6): 535–46. https://doi.org/10.1111/irv.12687
27. Munster V.J., de Wit E., Feldmann H. Pneumonia from human coronavirus in a macaque model. *N. Engl. J. Med*. 2013; 368(16): 1560–2. https://doi.org/10.1056/NEJMc1215691
28. de Wit E., Rasmussen A.L., Falzarano D., Bushmaker T., Feldmann F., Brining D.L., et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proc. Natl. Acad. Sci. USA.* 2013; 110(41): 16598–603. https://doi.org/10.1073/pnas.1310744110
29. Yao Y., Bao L., Deng W., Xu L., Li F., Lv Q., et al. An animal model of MERS produced by infection of rhesus macaques with MERS coronavirus. *J. Infect. Dis*. 2013; 209(2): 236–42. https://doi.org/10.1093/infdis/jit590
30. Falzarano D., de Wit E., Feldmann F., Rasmussen A.L., Okumura A., Peng X., et al. Infection with MERS-CoV causes lethal pneumonia in the common marmoset. *PLoS Pathogens*. 2014; 10(8): e1004250. https://doi.org/10.1371/journal.ppat.1004250
31. Sun SH, Chen Q., Gu H.J., Yang G., Wang Y.X., Huang X.Y., et al. A mouse model of SARS-CoV-2 infection and pathogenesis. *Cell Host Microbe*. 2020; 28(1): 124–133.e4. https://doi.org/10.1016/j.chom.2020.05.020
32. Lu R., Zhao X., Li J., Niu P., Yang B., Wu H., et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020; 395(10224): 565–74. https://doi.org/10.1016/S0140-6736(20)30251-8
33. Bao L., Deng W., Huang B., Gao H., Liu J., Ren L., et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature*. 2020; 583(7818): 830–3. https://doi.org/10.1038/s41586-020-2312-y
34. Hassan AO, Case J.B., Winkler E.S., Thackray L.B., Kafai N.M., Bailey A.L., et al. A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. *Cell*. 2020; 182(3): 744–53.e4. https://doi.org/10.1016/j.cell.2020.06.011
35. Israelow B., Song E., Mao T., Lu P., Meir A., Liu F., et al. Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. *J. Exp. Med.* 2020; 217(12): e20201241. https://doi.org/10.1101/2020.05.27.118893
36. Ng S.C., Tilg H. COVID-19 and the gastrointestinal tract: more than meets the eye. *Gut*. 2020; 69(6): 973–4. https://doi.org/10.1136/gutjnl-2020-321195
37. Xu Y., Li X., Zhu B., Liang H., Fang C., Gong Y., et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat. Med*. 2020; 26(4): 502–5. https://doi.org/10.1038/s41591-020-0817-4
38. Sia S.F., Yan L.M., Chin A.W.H., Fung K., Choy K.T., Wong A.Y.L., et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature*. 2020; 583(7818): 834–8. https://doi.org/10.1038/s41586-020-2342-5
39. Chan J.F., Zhang A.J., Yuan S., Poon V.K., Chan C.C., Lee A.C., et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin. Infect. Dis*. 2020; ciaa325. https://doi.org/10.1093/cid/ciaa325
40. Lau S.Y., Wang P., Mok B.W., Zhang A.J., Chu H., Lee A.C., et al. Attenuated SARS-CoV-2 variants with deletions at the S1/S2 junction. *Emerg. Microbes Infect*. 2020; 9(1): 837–42. https://doi.org/10.1080/22221751.2020.1756700
41. Imai M., Iwatsuki-Horimoto K., Hatta M., Loeber S., Halfmann P.J., Nakajima N., et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl. Acad. Sci. USA*. 2020; 117(28): 16587–95. https://doi.org/10.1073/pnas.2009799117
42. Rogers T.F., Zhao F., Huang D., Beutler N., Burns A., He W.T., et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science*. 2020; 369(6506): 956–63. https://doi.org/10.1126/science.abc7520
43. Shi J., Wen Z., Zhong G., Yang H., Wang C., Huang B., et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. 2020; 368(6494): 1016–20. https://doi.org/10.1126/science.abb7015
44. Kim Y.I., Kim S.G., Kim S.M., Kim E.H., Park S.J., Yu K.M., et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. *Cell Host Microbe*. 2020; 27(5): 704–9.e2. https://doi.org/10.1016/j.chom.2020.03.023
45. Park S.J., Yu K.M., Kim Y.I., Kim S.M., Kim E.H., Kim S.G., et al. Antiviral efficacies of FDA-approved drugs against SARS-CoV-2 infection in ferrets. *mBio*. 2020; 11(3): e01114-20. https://doi.org/10.1128/mBio.01114-20
46. Melin A.D., Janiak M.C., Marrone F., Arora P.S., Higham J.P. Comparative ACE2 variation and primate COVID-19 risk. *bioRxiv.* 2020; 2020.04.09.034967. Preprint. https://doi.org/10.1101/2020.04.09.034967
47. Singh D.K., Ganatra S.R., Singh B., Cole J., Alfson K.J., Clemmons E., et al. SARS-CoV-2 infection leads to acute infection with dynamic cellular and inflammatory flux in the lung that varies across nonhuman primate species. *bioRxiv*. 2020. https://doi.org/10.1101/2020.06.05.136481
48. Rockx B., Kuiken T., Herfst S., Bestebroer T., Lamers M.M., Oude Munnink B., et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science*. 2020; 368(6494): 1012–5. https://doi.org/10.1126/science.abb7314
49. Lu S., Zhao Y., Yu W., Yang Y., Gao J., Wang J., et al. Comparison of SARS-CoV-2 infections among 3 species of non-human primates. *bioRxiv*. 2020. https://doi.org/10.1101/2020.04.08.031807
50. Bao L., Deng W., Gao H., Xiao C., Liu J., Xue J., et al. Lack of reinfection in rhesus macaques infected with SARS-CoV-2. *bioRxiv.* 2020. https://doi.org/10.1101/2020.03.13.990226
51. Maisonnasse P., Guedj J., Contreras V., Behillil S., Solas C., Marlin R., et al. Hydroxychloroquine in the treatment and prophylaxis of SARS-CoV-2 infection in non- human primates. *Nature*. 2020; 585(7826): 584–7. https://doi.org/10.21203/rs.3.rs-27223/v1

Information about the authors

Aleksey M. Nagornykh✉ — Cand. Sci. (Vet.), researcher, Scientific group of experimental models' development, Laboratory of experimental pharmacology, Department of molecular diagnostics and epidemiology, Central Research Institute of Epidemiology, 111123, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0002-8320-2999.
E-mail: nagornih@cmd.su

Aleksander I. Tyumentsev — Cand. Sci. (Biol.), Head, Laboratory of experimental pharmacology, Department of molecular diagnostics and epidemiology, Central Research Institute of Epidemiology, 111123, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0003-0537-2586.
E-mail: tymencev@cmd.su

Marina A. Tyumentseva — Cand. Sci. (Biol.), Head, Laboratory of genome editing, Department of molecular diagnostics and epidemiology, Central Research Institute of Epidemiology, 111123, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0002-3145-3702.
E-mail: tyumentseva@cmd.su

Vasily G. Akimkin — D. Sci. (Med.), Professor, Full Member of the Russian Academy of Sciences, Director, Central Research Institute of Epidemiology, 111123, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0003-4228-9044.

Contribution: the authors contributed equally to this article.

Информация об авторах

Нагорных Алексей Михайлович✉ — к.вет.н., н.с. научной группы разработки экспериментальных моделей лаб. экспериментальной фармакологии отдела молекулярной диагностики и эпидемиологии ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, 111123, Москва, Россия.
ORCID ID: https://orcid.org/0000-0002-8320-2999.
E-mail: nagornih@cmd.su

Тюменцев Александр Игоревич — к.б.н., зав. лаб. экспериментальной фармакологии отдела молекулярной диагностики и эпидемиологии ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, 111123, Москва, Россия.
ORCID ID: https://orcid.org/0000-0003-0537-2586.
E-mail: tymencev@cmd.su

Тюменцева Марина Алексеевна — к.б.н., зав. лаб. геномного редактирования отдела молекулярной диагностики и эпидемиологии ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, 111123, Москва, Россия.
ORCID ID: https://orcid.org/0000-0002-3145-3702.
E-mail: tyumentseva@cmd.su

Акимкин Василий Геннадиевич — д.м.н., проф., акад. РАН, директор ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, 111123, Москва, Россия.
ORCID ID: https://orcid.org/0000-0003-4228-9044.

Участие авторов: все авторы сделали эквивалентный вклад в подготовку публикации.