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Investigation of SARS-CoV-2 Infection in Domestic Animals

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Abstract

Concerned with the COVID-19 pandemic, the study of this disease in animals has got a great scientific importance in clarifying the information about the source and circulation of the infection. The study aimed to investigate the source of infection of domestic animals (dog, cat, cattle, sheep, goat and poultry) with SARS-CoV-2, as well as to identify susceptible animal species and ways of transmission of the virus. Observations were made on the animals selected for the study, from which nasopharyngeal and oropharyngeal smears were taken for PCR, and blood samples were taken for enzyme-linked immunosorbent assay (ELISA).

The experimental part of the study was carried out in veterinary clinics, animal shelters and farms. Dogs and cats are kept in animal shelters and examined in veterinary clinics, as well as cattle, sheep, goats and poultry grown on various farms, were involved in the study.

Antibodies to SARS-CoV-2 were detected in 11 of 645 samples taken from animals whose clinical signs of COVID-19 disease were initially observed or whose owners were exposed to the disease.

Based on the results of the study, monitoring the dynamics of the spread of SARS-CoV-2 among animals is of great scientific and practical importance in preventing this process.

Keywords: Coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), investigation, antibody, domestic animals.

Introduction

SARS-CoV-2 infection, caused the COVID-19 pandemic in the world and atypical pneumonia in humans, was first detected in late 2019 in the Chinese city of Wuhan and was named 2019-nCoV (Shchelkanov et al., 2020). The genome structure of the causative agent is homologous to 50% MERS-CoV, 79% SARS-CoV and 88% BtRsCoV, so it belongs to the second type of acute respiratory syndrome viruses (Pal et al., 2020). According to recent data, SARS-CoV-2 is classified as a highly mutagenic virus belonging to the Beta- and Deltacoronavirus groups of the *Orthocoronavirinae* subfamily of the *Coronaviridae* family (Zhou et al., 2020).

Although the exact source of the causative agent of infection is not known, it is believed that SARS-CoV-2 was originated in wild animals and, later, transmitted to humans (Ahmad et al., 2020). According to preliminary data, the spread of the disease was caused by bats sold at animal markets in the Chinese city of Wuhan (Mackenzie & Smith, 2020). In the later stages of the pandemic, rodents were also reported to be infected with Sars-CoV-2 in various parts of the world. Rodents are thought to have played an intermediate role in transmitting the virus from bats to humans (Yuan et al., 2020).

Although there are no solid scientific findings on the mechanism of transmission of SARS-CoV-2 from animals to humans or vice versa, in some countries, owners of dogs and cats infected with SARS-CoV-2 have been found to have the disease (Sit et al., 2020; Calvet et al., 2021). In the United States, there have also been reports of the disease being transmitted to zoo animals through contact with an employee infected with SARS-CoV-2 (USDA, 2020). In late April 2020, Netherlands reported the first case of SARS-CoV-2 infection in commercially farmed mink. Additional reports of SARS-CoV-2 infected farmed mink came later in 2020 from Denmark (June), Spain (July), the U.S. (August), Italy (August), Sweden (October), France (November), Greece (November), Lithuania (November), and Canada (December) (AVMA, 2021).

Material and methods

Dogs and cats are kept in animal shelters and examined in veterinary clinics, as well as cattle, sheep, goats and poultry grown on various farms, were involved in the study. At the initial stage of the study, blood samples were taken from animals (nasopharyngeal and oropharyngeal smears) for molecular genetic (PCR) examinations, and blood from peripheral veins for enzyme-linked immunosorbent assay (ELISA) examinations.

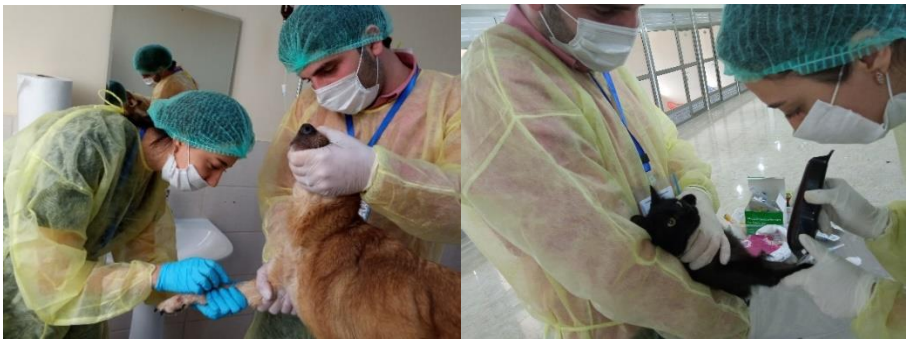

Figure 1. Blood sampling procedure

Samples were taken from 211 dogs, 136 cats, 19 cattle, 268 ruminants (sheep, goats) and 11 chickens. PCR and ELISA tests (virus in swab samples, antibodies in blood samples against the virus) were performed on samples taken from dogs and cats, and ELISA tests (antibodies in blood samples) were performed on samples taken from other animals. Blood samples were collected via leg venipuncture and sera were separated and stored at −20°C until further processing. All samples were collected under full personal-protective equipment.

Molecular genetic analyzes were performed using the BIO-RAD CFX96 Real-Time device. Extraction process done by the QIAamp® Viral RNA Mini kit. During the Extraction process, 140µl of the sample was taken at the initial stage and 160µl of RNA was isolated. Purification carried through Oasig™ lyophilized OneStep 2X RT-qPCR Master Mix kit, 5µl of extracted RNA was added to 20µl of Master mix. In the last stage, 25µl of the mixture was placed on the device for reading and recording.

Enzyme-linked immunosorbent assay (ELISA) analyzes were performed using the Thermo Multiskan FC device using the ID Screen SARS-CoV-2 Double Antigen Multi-species kit. Double antigen ELISA for the detection of antibodies directed against the nucleocapsid of SARS-CoV-2 in animal serum or plasma. Before the start

of analysis, all kit reagents were kept at room temperature (21°C± 5°C) and homogenized by vortexing. Firstly, 25µl Dilution buffer was added to each well. Secondly, an equal amount of Negative and Positive control was added properly to wells A1 and B1, C1 and D1. Then, 25µl sample was added to each remaining wells. The plate was covered and placed in a thermostat for 45 minutes of incubation. The plate was emptied and washed 3 times with 300µl wash solution. Then 300µl conjugate was added to each well. The plate was covered and kept at room temperature for 30 minutes. Again, the plate was emptied and washed 3 times with 300µl wash solution. In the next step, 100µl of substrate solution was added. The plate was covered and kept in the dark condition at room temperature for 20 minutes. In the end, 100µl of stop solution was added. The results were read and recorded at 450 nm.

Result and discussion

It should be noted that no SARS-CoV-2 agent was detected in the smear samples as a result of the research. No antibodies to SARS-CoV-2 were detected in immunosorbent assays analysis in blood samples from cattle, sheep, goats, and poultry. However, blood samples taken from 7 dogs and 4 cats with symptoms of the disease (runny nose, cough, diarrhea, etc.) revealed the presence of antibodies against the virus. The results demonstrate that the disease is limited among dogs and cats. Information on the type, age, residential area (owner/stray), and the amount (titer) of specific antibodies in the blood of dogs and cats, for which antibodies to SARS-CoV-2 have been detected, are presented in Tables 1 and 2.

Table 1. The table provides information on dogs with ≥60% antibodies in their blood.

Dog Nº	Gender of animal	Age	Sampling place	Titer (%)
1	Male	10 months	Animal shelter	143,58
2	Female	1 year	Animal shelter	157,18
3	Female	2 years	Animal shelter	64,24
4	Female	4 years	Vet. clinic	61,00
5	Female	3,5 months	Vet. clinic	237,86
6	Male	2 years	Vet. clinic	60,23
7	Male	1 years	Vet. clinic	98,97

As can be seen, the number of dogs with positive results is 7(Dog numbers are conditional). Of these, 62.24% of titers were found in the 3rd dog, 61.00% in the 4th dog, and 60.23% in the 6th dog. The antibody titer is 98.97 in the 7th dog. The 1st, 2nd and 5th dogs were found to have the highest titers, which are 143.58%, 157.18% and 237.86%, respectively. (≥ 60% positive) *

Table 2. The table provides information on cats with ≥60% antibodies in their blood.

Cat Nº	Gender of animal	Age	Sampling place	Titer (%)
1	Female	2 years	Vet. clinic	104,39
2	Female	3 years	Vet. clinic	162,19
3	Male	1,5 months	Vet. clinic	226,58
4	Female	8 months	Vet. clinic	153,63

As can be seen, the number of dogs with positive results is 4(Cat numbers are conditional). Of these, 104.39% of titers were found in the 1st cat, 162.19% in the 2nd cat, 226.58% in the 3rd and 153.63% in the 4th dog. (≥ 60% positive) *

* According to the instructions of the ID Screen SARS-CoV-2 Double Antigen Multi-species set, the result is considered positive if the antibody titer is ≥ 60%.

347 samples were examined by PCR and the result was negative. This means that no active patients were recorded in the sampled dogs and cats.

The results of the study show that dogs and cats are more susceptible to the disease among experimental animals, and the incidence of the disease among dogs is higher than among cats. Thus, the incidence was 3.32% among dogs and 2.94% among cats.

At the same time, the incidence of SARS-CoV-2 infection in females, both dogs and cats, is high, and the incidence of disease in domesticated animals is predominant (73%). Given the direct contact of dogs with antibodies in the blood of animals living in shelters, it can be assumed that there is a risk of transmission of the coronavirus from animal to animal.

The level of antibody titer, which is important in the formation of immunodeficiency, is inversely proportional to the age of the animals in the dog population, and the

amount of titer produced decreases with age. In cats, however, it can be concluded that the antibody titer in the blood is not related to the age of the animal.

During the collection of survey data on infected animals, it was determined that there were people infected with COVID-19 or with clinical symptoms of the disease among the owners of the animals or the staff serving them in the shelters. This, in turn, indicates that the human factor plays a role in the circulation of the pathogen in the transmission of the pathogen to animals.

Our results including 7 dogs and 4 cats with symptoms of the disease (runny nose, cough, diarrhea, etc.) revealed the presence of antibodies against the virus.

Since no antibodies to SARS-CoV-2 or the pathogen have been found in cattle and ruminants (cattle, sheep and goats), as well as poultry, it can be concluded that these animals are insensitive to the disease. Cases of SARS-CoV-2 infection have been reported in domestic dogs and cats. Thus, the formation of specific antibodies in the blood of domestic animals was observed by immunosorbent assays. Exposure of animals to SARS-CoV-2 infection increases the likelihood of contact with their infected owners or infected caregivers. Therefore, while contacting with animals during the COVID-19 pandemic, people should strictly follow the sanitary and hygienic rules and take into account the possibility of cross-transmission of zoonotic SARS-CoV-2. Research should also be continued to investigate the possibility of the virus circulating among animals and transmitting it from animals to humans.

More studies are needed to investigate the transmission route of SARS-CoV-2 from humans to cats and dogs. Finally, it is imperative that further studies be quickly carried out in order to better establish the risk of contamination of pets from humans, as well as the risk that infected pets would have as a source of infection for humans. Importantly, immediate action should be implemented to keep a suitable distance between humans and pet animals such as cats and dogs, and strict hygiene and quarantine measures should also be carried out for these animals.

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