INSTITUTO POLITÉCNICO
DE VIANA DO CASTELO

Andreia Sofia Teixeira de Oliveira

SEROPREVALENCE OF SARS-CoV-2 IN PET-OWNED CATS FROM PORTUGAL

Veterinary Nursing in Small Animals Master

This work was carried out under the orientation of
Professor Helena Vala
Professor Maria Aires Pereira
Professor Teresa Letra Mateus

August 2022

The doctrines presented in this thesis are the exclusive responsibility of the author.

Table of Contents

Acknowledgements

This chapter is equally important as all the others that compose this Master's dissertation, since I could not forget to thank all the people and entities that helped me on this journey.

First of all, to my parents, who encouraged me to do the Master's degree from the beginning.

To my friends and colleagues, for their help and motivation. Without them, the performance of this study would definitely be very challenging to be carried out. Susana Costa, Clara Barbosa, Henrique Silva, Tiago Abreu, Sandrina Vieira, Ana Catarina Santos, Daniela Pinto, Cristiana Silva and many others that I cannot list. To all of them, thank you very much!

To my advisor Helena Vala and co-advisors Teresa Letra Mateus and Maria Aires Pereira, for their patience, kindness, understanding and availability. Without them, this project would not be at all possible.

To my colleagues at the Hospital Veterinário de Gaia, for their help and immediate disposability in taking the samples. To all the other clinics and veterinary hospitals in the country that immediately accepted to cooperate in the study, I am eternally grateful!

And last but not the least, to my pets, Sansão and Tucha, for the company they made me during this period, while I wrote the dissertation.

“Para ser grande, sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa. Põe quanto és
No mínimo que fazes.
Assim em cada lago a lua toda
Brilha, porque alta vive.”
- Fernando Pessoa

ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the agent responsible for the infectious disease named COVID-19, the most recent pandemic disease. This dissertation aims to focus on the epidemiology of the virus, including the impact under the concept of "One Health" and description of preventive measures to be taken, namely in animal health promotion, as well as investigate anti-SARS-CoV-2 IgG seroprevalence in client-owned cats from Portugal and evaluate the infection risk of cats that maintain contact with human COVID-19 cases. The first human case was reported in Wuhan, China, in late 2019, and bats were the first hosts considered the vehicle of transmission of this virus, however, the possible animal origin of the new Coronavirus has not been yet confirmed. The close contact between humans and domestic cats raises concerns about the potential risks of SARS-CoV-2 transmission. The first case described in animals was a cat that tested positive for SARS-CoV-2 in March 2020, in China. After that, several cases in different animal species have already been identified around the world, in Europe and even in Portugal. In order to understand the potential infection for SARS-CoV-2 in animals, a total of 176 cats were sampled. Cat owners answered an online questionnaire and cats were screened for antibodies against SARSCoV-2, using a commercial ELISA. Twenty (21.3%) households reported at least one confirmed human COVID-19 case. Forty cats (22.7%) belonged to a COVID-19 positive and 136 (77.3%) to a COVID-19 negative household. The seroprevalences of cats from COVID-19 positive and negative households were 5.0% (2/40) and 0.7% (1/136). The two SARS-CoV-2-seropositive cats from COVID-19-positive households had an indoor lifestyle, and their owners maintained a close and frequent contact with them, even after being diagnosed with COVID-19, pointing towards human-to-cat transmission. The SARS-CoV-2-seropositive cat from the COVID-19 negative household had a mixed indoor/outdoor lifestyle and chronic diseases. Owners of the three SARS-CoV-2-seropositive cats did not notice clinical signs or behavior changes. This results highlights the low risk of SARS-CoV-2 transmission from COVID-19 positive human household members to domestic cats, even in a context of close and frequent human–animal contact.

Keywords: coronavirus; felids; infection; epidemiology; One Health; zoonosis; anti-SARS-CoV-2 antibodies.

List of Abbreviations

ACE2- Angio-Tensin-Converting Receptor Enzyme 2

Alpha-CoV- Alpha Coronavirus

BatCoV- Bat Coronavirus

BCoV- Bovine Coronavirus

Beta-CoV- Beta Coronavirus

CCoV- Canine Coronavirus

CDCP- Centers for Disease Control and Prevention

CIRD- Canine Infectious Respiratory Pathology

Co- Company

CoV- Coronavirus

COVID-19- 2019 Coronavirus Disease

CRCoV- Canine Respiratory Coronavirus

Delta-CoV- Delta Coronavirus

ECoV- Equine Coronavirus

ELISA- Enzyme-Linked Immunosorbent Assay

ESAV- Escola Superior Agrária de Viseu

FCoV- Feline Coronavirus

FeLV- Feline Leukemia Virus

FIP- Feline Infectious Peritonitis

FIV- Feline Immunodeficiency Virus

Gamma-CoV- Gamma Coronavirus

GfCoV- Guineafowl Coronavirus

HCoV- Human Coronavirus

IBV- Infectious Bronchitis Virus

IgG- Immunoglobulin G

Ltd- Limited

ml- millilitres

MERS-CoV- Middle East Respiratory Coronavirus

MHV- Murine Hepatitis Virus

nCOV- novel Coronavirus

nm- nanometers

NS- Negative samples

OD- Optical density

PDCoV- Porcine Delta Coronavirus

PEDV- Porcine Epidemic Diarrhea

PhCoV- Faisant Coronavirus

PHEV- Porcine Haemagglutinating Encephalomyelitis

PS- Positive samples

PRCV- Porcine Respiratory Coronavirus

qPCR- quantitative Polymerase Chain Reaction

QCoV- Quail Coronavirus

RCF- Relative Centrifugal Force

RNA- Ribonucleic Acid

RT-PCR- Reverse Transcription Polymerase Chain Reaction

RT-qPCR - Reverse Transcription-quantitative Polymerase Chain Reaction

SADS-CoV- Swine Acute Diarrhoea Syndrome Coronavirus

SARS-CoV- Severe Acute Respiratory Syndrome Coronavirus

SARS-CoV-2- Severe Acute Respiratory Syndrome Coronavirus 2

TCoV- Turkey Coronavirus

TGEV- Transmissible Gastroenteritis Virus

USA- United States of America

WHO- World Health Organization

WOAH - World Organisation for Animal Health

WSAVA- World Small Animal Veterinary Association

ºC- Celsius degrees

®- registered brand

<- lower than

>- higher than

%- percent

±- more or less

= - equal

List of Tables

List of Figures

CHAPTER I - LITERATURE REVIEW

1.1. Introduction

In December 2019, cases of pneumonia of unknown origin were reported in the city of Wuhan, China. A novel coronavirus (2019-nCoV) was identified as the causal agent of the disease by the Chinese authorities [1] and the disease officially designated 2019 Coronavirus Disease (COVID-19) was classified by the World Health Organization (WHO) as a pandemic [2]. Afterwards, with the new virus being responsible for the second coronavirus caused acute respiratory syndrome, the virus designation changed to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [3]. The first reports of human COVID-19 outbreaks were attributed to the consumption of wild animals, traded in markets, known as "wet markets". The WHO did not specifically confirmed this source, however the diversity of species susceptible to SARS-CoV and SARS-CoV-2 related viruses, highly suggests a propensity for these viruses to cross the species barrier, particularly in the context of frequent interactions between carnivores and other small mammals, which may also facilitate transmission between species [4].

Domestic cats (*Felis catus*) are popular companion animals, being present in half of Portuguese households [5]. The amount of contact between domestic cats and humans varies but can be as close as sleeping in a bed and licking the owner's face [6].

Experimental infection of domestic cats has shown that they are susceptible to SARS-CoV-2 infection, ensuring virus replication [7]. Following the infection, the cat may stay asymptomatic or manifest respiratory pathology [8,9].

According to current knowledge, cats are mainly infected through contact with infected owners (reverse zoonosis) [10,11]. However, transmission from humans to cats appears to be an occasional event that does not always occur [12]. Transmission between cats has also been documented [13]. By mentioned reasons, this animal species should be included in animal and public health topic [14].

There are still gaps in the knowledge of this disease due to recent appearance of this zoonotic coronavirus [15]. However, high contagiousness and pathogenicity of this agent, as well as its potential zoonotic transmission, are well established topics [16,17], mainly due to the structural plasticity of the spike protein and the epidemiological, biological and virological characteristics of coronaviruses [18].

Although natural transmission of SARS-CoV-2 in companion animals has been reported, studies on this issue are still limited. Model animals for experimental transmission included cats, ferrets and hamsters [19].

Until now, this virus has suffered new variants, still resulting in high infection and mortality rates [17], reinforcing the crucial importance of accelerating studies, including the determination of the role of animals in transmission and potential reservoirs or vectors of the disease, which will provide future plans for risk analysis [3].

Thus, this research aims to study SARS-CoV-2 infection in cats from the One Health perspective, as well as the risk factors involved in infection at the owner-cat interface. Large-scale testing of susceptible species is needed to assess the extent of animal infection under natural breeding conditions. In this dissertation, we will also focus on current information on the zoonotic transmission of SARS-CoV-2 through direct and indirect contact between humans and the full range of farmed and wild animals and report on an epidemiological survey from November 2020 to October 2021 in cats living in mainland Portugal.

1.2. Coronaviruses

SARS-CoV-2 belongs to the family Coronaviridae, order Nidovirales [20], composed of two subfamilies, whose members are commonly referred to as CoV [21]. The CoVs have four genera, namely, *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* (Gamma-CoV) and *Deltacoronavirus* (delta-CoV), where bats, birds and rodents are considered to be natural reservoirs [1]. *Alphacoronavirus* (alpha-CoV) are agents responsible for porcine epidemic diarrhea (PEDV) and severe acute diarrhea; *Betacoronavirus* (beta-CoV) are responsible for porcine haemagglutinating encephalomyelitis (PHEV) [1,22].

The name CoV is derived from the Latin word corona, meaning crown, due to the distinctive structure of the virus, as the surface projections on the viral envelope resemble a crown [1,23].

Coronaviruses are positive-sense RNA viruses and RNA genome [24], capable of infecting humans, mammals and poultry [25]. Human coronavirus (HCoV) causes mild respiratory symptoms or can be asymptomatic, compared with severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory coronavirus (MERS-CoV) and SARS-CoV-2 which causes severe to lethal respiratory symptoms [23,26].

Several diseases with CoV origin are reported in Table 1.

Table 1- Coronavirus genus, disease name, abbreviation and hosts on different animals.

Genus	*Virus*	*Abbreviation*	*Host*	*First outbreak*	*Reference*
Alpha	Feline coronavirus	FCoV	Cat	1960s	[27]
	Transmissible gastroenteritis virus	TGEV	Pig	1970	[28]
	Porcine respiratory coronavirus	PRCV	Pig	1984	[29]
	Canine coronavirus	CCoV	Dog	2004	[30]
	Porcine epidemic diarrhoea virus	PEDV	Pig	1977	[29]
	Swine acute diarrhoea syndrome-coronavirus	SADS-CoV	Pig	2017	[31]
	Bat coronaviruses	Bat CoV	Bat	2002	[32]
Beta	Bovine coronavirus	BCoV	Cow	1980	[33]
	Canine respiratory coronavirus	CRCoV	Dog	2003	[34]
	Equine coronavirus	ECoV	Horse	2000	[35]
	Porcine hemagglutinating encephalomyelitis virus	PHEV	Pig	1962	[87]
	Murine hepatitis virus	MHV	Murine	1947	[29]
	Middle East respiratory syndrome-related coronavirus	MERS-CoV	Camel	2012	[37]
	Severe acute respiratory syndrome coronavirus	SARS-CoV	Bat	2002	[38]
	Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2	Bat	2019	[13]
	Bat coronaviruses	Bat CoV	Bat	2002	[32]
Gamma	Infectious bronchitis virus	IBV	Avian	1930s	[39]
	Turkey coronavirus	TCoV	Turkey	1951	[40]
Delta	Porcine deltacoronavirus	PDCoV	Pig	2012	[29]

Epidemiological, biological and virological characteristics of coronaviruses are suggestive that contamination of animals by infected owners is highly probable but equally expected, due to the numerous opportunities to contact during an outbreak [21].
To support virus replication, CoVs use host intermediates to adapt the human receptors. In 2017, studies identified human SARS-CoV-like viral cells in bats, demonstrating the ability to infect humans. More than 60 chiropteran CoVs capable of infecting cultured human cells have also been identified [41].
In poultry, a few other types of coronavirus infection exist, such as Turkey Coronavirus (TCoV), Quail Coronavirus (QCoV), Guineafowl Coronavirus (GfCoV) and Faisant Coronavirus (PhCoV) [42]. Clinical presentations of infectious bronchitis virus (IBV), also an avian coronavirus, include respiratory and reproductive diseases. The determining

factors for the development of these symptoms depends on the environmental features of the birds, nutritional factors, age and genetic background [43].

Porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) are alphacoronaviruses responsible for gastrointestinal disorders in pigs, such as porcine acute diarrhea coronavirus syndrome (SADS-CoV). Pig deltacoronavirus (PDCoV) also causes diarrhea, vomiting, anorexia and death in newborns [43]. There is another porcine alphacoronavirus that doesn't affect the gastrointestinal tract but the respiratory tract: porcine respiratory coronavirus (PRCV) [44].

In terms of neurological and/or digestive pathologies, porcine haemagglutinating encephalomyelitis virus (PHEV) is the responsible agent [44].

These animals are potential reservoirs of SARS-CoV and SARS-CoV-2 [42], however, according to Meekins et al. [45], none of the studies showed any virus transmission to other healthy pigs.

Cattle, goats, sheep, buffalo, alpacas and llamas were found to be infected with Bovine Coronavirus (BCoV) which also induces respiratory pathology [42]. This virus had potential to induce severe clinical disease in several age groups of cattle, namely enteric disease in calves, enteric disease in adult cattle and respiratory disease at other age stages [43,44,46].

Domestic and wild felids are the carriers of Feline Coronaviruses (FCoVs), represented by two distinct types: FCoV type I, the most common circulating in domestic cat reservoirs and FCoV type II, the least prevalent epidemiologically [47].

One of the most important viral diseases in cats caused by a coronavirus is Feline Infectious Peritonitis (FIP). Clinical signs include ocular and neurological signs. In the progressive form of FIP, there is abdominal distension and pleural effusion, and can result in the death of the animal in a period of weeks to months [43]. The World Small Animal Veterinary Association (WSAVA) Vaccination Guidelines Group has provided veterinary health professionals with information on the use of the FIP vaccine, however, there is no scientific basis to support that it induces immunity in cats against SARS-COV-2.

Canine alphacoronavirus coronavirus (CCoV) causes enteric infection [48] by CCoV type I or/and CCoV type II [49]. CCoV type I was discovered to be responsible for enteritis in puppies. This virus subsequently mutated, resulting in the variant CCoV type

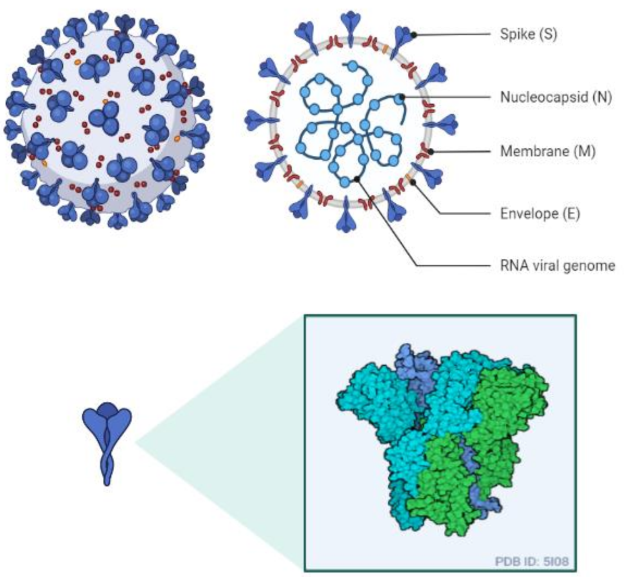
II [50]. Beta-coronavirus, is considered to be the etiological agent of Canine Infectious Respiratory Pathology (CIRD) [44].

1.3. Phylogeny and Genomics of SARS-CoV-2

According to the World Organisation for Animal Health (WOAH) [51], COVID-19 is defined as an infectious disease caused by a new discovered coronavirus, SARS CoV-2. Coronavirus (CoV) are large single-stranded positive-sense RNA viruses with high mutation rates, after replicating, and also known as versatile and variable viruses [52].
Structurally, the virus contains four proteins, the membrane (M), envelope (E), spike (S) and nucleocapsid (N) proteins. The N and S proteins are responsible for the development of specific antibodies (Figure 1a) [53].
SARS-CoV-2 spike proteins S is similar to the other coronavirus spike proteins and is a type I membrane glycoprotein designed in the shape of a trimer (Figure 1b) [17].
According to Zhang et al. [54], S protein binds to the angio-tensin-converting receptor enzyme 2 (ACE2) on a host cell and subtracts major structural rearrangements to promote membrane fusion.

a)

b)



Figures 1- a) Coronavirus structure; **b)** SARS-CoV-2 spike protein structure.

SARS-CoV-2 mutations help to understand the diversity of the variant and their impact on virulence and pathogenicity, since the SARS-CoV-2 protein interacts with several human proteins, such as CUL2, ELOB, ELOC, MAP7D1, PPT1, RBX1, THTPA, TIMM8B, and ZYG11B, found in lung tissue [55].

The actual SARS-CoV-2 carries the most infectious D614G mutation, providing an advantage for SARS-CoV-2 ability of transmission [56], however, this variant is not responsible for the severity of the disease, as the pathogenic mechanisms are more than just infectious, due in part to the increased ability for human-to-human transmission [57].

1.4. Epidemiology of SARS-CoV-2 in animals

1.4.1. Etiology and Epidemiology

The SARS-CoV-2 is similar to bat coronavirus by over 95% and similar to SARS-CoV by over 70% [58], which allows for increased transmission capacity between species [59]. According to Sharun et al. [60], the possibility that SARS-CoV-2 had origin in bats exists, since this virus contains genomic characteristics similar to bat-CoV and considering also the greatest natural reservoir host of bat for virus in general, as with the rabies virus and many others.

The prevalence of SARS-CoV infection has been high in farm animals, used for human consumption, such as masked palm civets (*Paguma larvata*), regarded as transitory incidental hosts and potential direct sources of human infection [61].

The nearest non-human primate to develop SARS-CoV-2 infection is rhesus macaques. Being similar to humans in terms of phylogeny, these animals are an important link and model for tests and development of vaccines against COVID-19 studies [62]. The spread of SARS-CoV-2 in the natural habitat of wild animals carries negative implications, especially for endangered species [63]. The Table 2 lists several animals' species and their location, infected by COVID 19 from 2020 to 2022, and Figure 2 illustrate the Europe countries where the positive cases appeared, in that period.

Domestic cats are susceptible to being infected by SARS-CoV-1 and SARS-CoV-2 [64]. Consequently, they become reverse transcription polymerase chain reaction (RT-PCR) positive and develop serum antibodies [65]. China was the country where the first case of a cat testing positive for COVID-19 was found [66].

Table 2- List of animals infected with SARS-CoV-2, from 2020 to 2022, in several regions.

Year	Species	Region/Country	Number of positive animals	Reference
2020	Dog	Italy	1	[67]
		Hong Kong	1	[68,70]
			2	[69,71]
		North America	21	[10,72]
		Argentina	4	[10,72]
		Japan	4	[10,72]
		Denmark	1	[67]
2020	Cat	Belgium	1	[11]
			31	[10,72]
		Chile	3	[10,72]
		Brazil	1	
		Argentina	2	
		Japan	2	
		Germany	1	[11]
		Russia	1	
		Spain	1	[67]
		Switzerland	1	[67]
		France	2	[67]
		Belgium	3	[73]
		Hong Kong	4	[74,75]
		New York	15	[76]
2020	Lion	New York	3	[51]
			6	[76]
		Barcelona	4	[51]
2020	Tigers	New York	4	[51]
			5	[75,76]
2020	Nonhuman Primates	United States of America	2	[61]
		Netherlands	9	[75]
2020	Cougar	South Africa	1	[51]
2021	Primates	California	3	[51]
2022	Dog	Portugal	7	[13]
	Cat		15	

Figure 2- Map of the Europe with signage of infected animals with COVID-19. Subtitles: red (Dogs); orange (Cats); grey (Minks); blue (Lions).

1.4.2. Transmission of SARS-CoV-2

As above mentioned, some studies suggest that bats are the vehicle of transmission of SARS-CoV and MERS-CoV, which justifies to proceed with investigations to ascertain if it was the original host of the of the actual SARS-CoV-2 pandemic and its potential for transmitting the disease (Figure 3) [77].

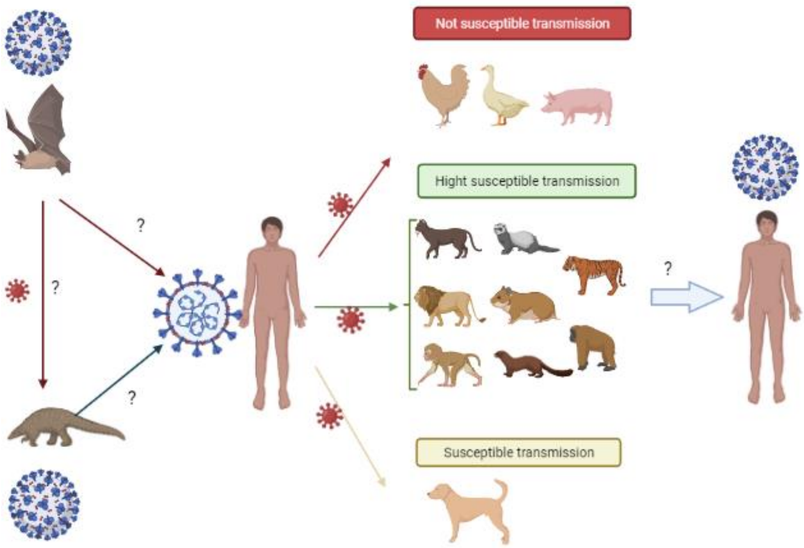


Figure 3- Transmission of SARS-CoV-2 in different species, through the bat.

1.4.2.1. Animal to animal transmission

Some CoV are capable to control some level of replication under specific environmental conditions, making them more receptive to their environments. In places with close health surveillance, such as zoos, the potential for SARS-CoV-2 to cross barriers is significantly reduced. In wildlife, close contact of multiple species is uncommon, making the spread of SARS-CoV-2 between animals being a challenge [78]. In poultry, for example, the transmission occurs from bird to bird (horizontal infection), and the potential of transmission through eggshells is being investigated [42].

1.4.2.2. Animal to human transmission

Until now, there is no evidence of zoonotic transmission between dogs and cats and humans, existing only one route of transmission well established, from human to animal, once all infected animals have had contact with humans with COVID-19 positive [65].

In mink farms, clinical data collected and samples taken from oral, nasal and rectal swabs, tissues and blood tests indicated that these animals are extremely sensitive to infection by the virus, presenting from asymptomatic states to sudden death [79]. These animals were the first species to infect humans and other animals that were highly susceptible to SARS-CoV-2 infection [45].

Frozen foods may represent a risk of SARS-CoV-2 transmission, as packages of cod, imported from China, showed signs of SARS-CoV-2 contamination. The importation and exportation, as well as the transport conditions of frozen and chilled fish, pose a threat of SARS-CoV-2 transmission [42]. There is still no evidence that cattle contributed to this transmission, but contact between infected people and animals causes anthropozoonotic infections [42].

1.4.2.3. Human to human transmission

SARS-CoV-2 is transmitted by several ways. Human transmission occurs by contact with contaminated fomites, with another infected person or through aerosols. In this case, droplets are expelled and penetrate the nasal, ocular and oral mucous membranes [80]. The virus, according to Parry [81] is also transmitted by faeces and contaminated water.

In humans, transplacental transmission in pregnant women infected with SARS-CoV-2 occurs when infectious agents break the placental barrier, causing vertical transmission (from mother to fetus), resulting in complications for both, while the virus enters the cells and reproduces, due to the presence of the SARS-CoV-2 virus in the amniotic fluid and fetal membranes, that may cause fetal death [82].

1.4.2.4. Human to animal transmission

The infection of animals with SARS-CoV-2 through direct contact with infected humans may have consequences for animal health and welfare, wildlife conservation and biomedical research. In the first mink farm infected with SARS-CoV-2, antibodies were detected in three of eleven cats, as a result of possible exposure to the virus from farm workers in the surroundings where the infected mink stayed [83].

Regarding wild big felines, kept in captivity, the fact of being reservoirs in contact with humans, the risk of infection from human to animal is notorious [84].

The first known domestic animals infected with SARS-CoV-2 were cats, dogs and ferrets, with cats and ferrets reported to be more susceptible to infection with SARS-CoV-2, compared to dogs [78]. In France, in a group of 22 cats, one was seropositive, confirmed by Enzyme-Linked Immunosorbent Assay (ELISA). These cats were from households of people suspected or confirmed to have SARS-CoV-2 infection [85]. In Portugal, between December 2020 and May 2021, 15 of 69 cats have tested seropositive to SARS-CoV-2, probably transmitted by their owners [13].

The first dog that tested positive for SARS-CoV-2 was a 17-year-old German Spitz whose owner had characteristic symptoms of COVID-19 infection. Saliva and nasal discharge samples were collected from the dog in three days and analyzed by qPCR, showing small amounts of viral RNA in the three samples, indicating a relatively low viral load. However, serological screening revealed the presence of SARS-CoV-2 antibodies, indicating that the animal was infected with the virus and developed a specific immune response [21]. In July 2020, three free-roaming adult male dogs fed by the community, were tested for SARS-CoV-2, without any sign of infection. Two of them tested positive for at least two SARS-CoV-2 gene targets N1 and N2, by RT-qPCR. However, this study was performed during the actual pandemic and there is no information about the infectious period between human and animal diagnosis [86].

1.4.3. Symptoms

1.4.3.1. Exotic and wild animals

Wild populations of great apes have been constantly threatened by respiratory tract pathologies, infected through humans. A group of Gorillas at a Zoo in San Diego presented coughing and nasal discharge and one of them developed pneumonia [87]. When infected with COVID-19, rhesus macaques show signs of pulmonary edema with an irregular breathing standard. Lung histopathology showed hyaline membranes, a finding not so recurrent in other experimental animals' models [62].

Raccoon dogs were considered an interesting animal for SARS-CoV-2 infection investigation for developing subclinical acute infection with involvement of upper respiratory tract and for their ability to transmit SARS-CoV-2 infection to other raccoon dogs [45].

In exotics, such as ferrets, clinical signs observed during SARS-CoV-2 infection were anorexia and fever [42]. Murine hepatitis virus (MHV) can induce hepatitis, enteritis and neurological diseases, specifically in rodents [43]. However, according to Meekins et al. [45] and Clayton et al. [15], not having a large number of people keeping ferrets as pets, makes them less susceptible to control and concern. However, is recommendable avoiding contact of infected people with them. Hamsters are also characterized as a promising animal model for studies on SARS-CoV-2 infection, since, among the animal species analyzed, all presented moderate to severe clinical signs, and may also be targets of terminal acute disease, with respiratory tract disorders [45].

There are no reports yet on natural infections between rabbits and humans, since there is no evidence of clinical signs during a possible infection [45].

In South Africa, at 2020, two pumas showed gastrointestinal and respiratory signs. One year after, three lions suffered respiratory signs. Viral RNA was detected in the upper respiratory tract and in the faeces of both animals [84]. As well, six lions and three tigers tested positive for COVID-19, possibly infected by their handler [86].

1.4.3.2. Poultry

Infectious bronchitis virus affects the respiratory, urinary and reproductive tracts of poultry, resulting in decreased production and egg quality [42,87].

1.4.3.3. Cats

In cats, the virus is able to multiply and replicate in the upper respiratory tract, causing respiratory pathology [15], as well as gastrointestinal signs, like diarrhea and vomiting [89].

Recent studies suggest that cats previously infected with SARS-CoV-2 develop only partial immunity to the virus but do not transmit the virus to other healthy cats [85].

1.4.3.4. Dogs

In the opposite of cats, dogs have low susceptibility to SARS-CoV-2 infection. Until now, there is no proof of disease transmission among dogs, even though there is limited viral replication in infected dogs [45]. In Spain, 40 dogs with clinical pulmonary signs were tested for SARS-CoV-2 together with 20 presumed healthy dogs. Antibodies to COVID-19 were discovered in 5 healthy dogs and in one sick dog. These dogs lived in the households of people who were positive for the infection with COVID-19 [85]. In Portugal, according to Barroso et al. [13], a study showed that 7 out of 148 dogs were seropositive, probably associated to positivity for COVID-19 in owners. Clayton et al. [15] reports that Beagles infected with SARS-CoV-2 showed poor replication of the virus in major organs and tissues, resulting in equally poor transmission between them.

1.5. Diagnosis of SARS-CoV-2 in animals

Although, the first diagnostic studies to detect SARS-CoV-2 were focused on humans, nowadays we can study and detect the infection in animals through serological and molecular assays [13].

According to the Centers for Disease Control and Prevention (CDCP) [90], tests for SARS-CoV-2 in animals are available on the market, however, it is the health authority who decides when it is necessary to test the animals. Although RT-PCR is a highly sensitive test method, according to Parry [81], a positive result only indicates the presence of viral nucleotides in the sample, not an active infection [91].

1.6. One Health concept

The concept of One Health, according to Sellars et al. [92], is "a policy framework that seeks to promote human, non-human and environmental health simultaneously", in other words, with the outbreak of the SARS-CoV-2 pandemic, health paradigms have been changed, so health cannot be addressed only to humans but also to the interrelation between humans, animals, plants and the environment [93].

According to Jørgensen and Das Neves [94], about 75% of emerging infectious diseases come from wild animals, being the bats the source of zoonotic and pandemic viruses such as Ebola, all SARS and MERS, among others.

The knowledge and control of new zoonotic infections, together with an implementation of a greater One Health approach, is crucial for the early treatment of these pathological conditions [60].

1.7. Study purposes

The close contact between humans and domestic cats raises concerns about the potential risks of SARS-CoV-2 transmission. Thus, this study aims to investigate anti-SARS-CoV-2 IgG seroprevalence in client-owned cats from Portugal and determine the infection risk of cats that maintain contact with human COVID-19 cases.

CHAPTER II - MATERIAL AND METHODS

2.1. Animal Recruiting and Sampling

Convenience sampling was used to select veterinary centers (clinics and hospitals) for this investigation as we were living a pandemic and with long periods of confinement and conditional travel. Eighteen veterinary centers from mainland Portugal were invited by email to participate in this study. Veterinary centers (8/18) that agreed to collaborate, received detailed instructions for sample collection and storage, informed consent (Appendix I), and a link to get access to an online questionnaire for owners.

During health care visits, veterinary practitioners from collaborating veterinary centers invited cat owners to participate in the study. Cat owners who agreed to collaborate, answered an online questionnaire designed to collect background information. Blood samples were collected according to veterinary norms into dry tubes and then centrifugated at 500 Relative Centrifugal Force (RCF) for 10 minutes. Supernatants were transferred to a 2 millilitres (mL) microtubes and stored at −20 degrees Celsius (◦C) until sent to Escola Superior Agrária de Viseu (ESAV) laboratory. Sample collection took place between November 2020 and October 2021.

2.2. Background Data Collection

A questionnaire was developed using an online platform (Google Forms® , Google LLC, Mountain View, California, United States) to collect data from each cat and household. The questionnaire was prepared in Portuguese language and consisted of 29 questions, of which 27 were closed-ended (dichotomic, multiple choice) and two were openended. The questionnaire covered five main topics, specifically: characterization of households, including human and animal elements and human–animal interaction (11 questions); characterization of sampled cats, including full signalment, lifestyle, prophylactic, and medical history (13 questions); and diagnoses of COVID-19 in cat owner and/or in other human members of the household (2 dichotomic questions, Yes/No). If respondents answered Yes to one of these two questions, they would answer questions on another topic designed to characterize COVID-19 diagnoses and interaction with the cat during the period of isolation (three questions). For internal validation, the questionnaire was evaluated by the authors (Appendix II).

2.3. Detection of anti-SARS-CoV-2 IgG antibodies

Serum samples were screened for antibodies against the nucleocapsid (N protein) of SARS-CoV-2 using a commercial and already validated multi-species indirect ELISA (figure 4a), according to manufacturer instructions. Reading was performed at a wavelength of 450 nanometers (nm) on a microplate reader MB 580 (Heales, Shenzhen Huisong Technology Development Co., Ltd, China) (Figure 4b).

(a) (b)

Figure 4- a) ID screen SARS-CoV-2 Double Antigen Multi-Species; **b)** Microplate reader.

2.4. Data Processing and Statistical Analysis

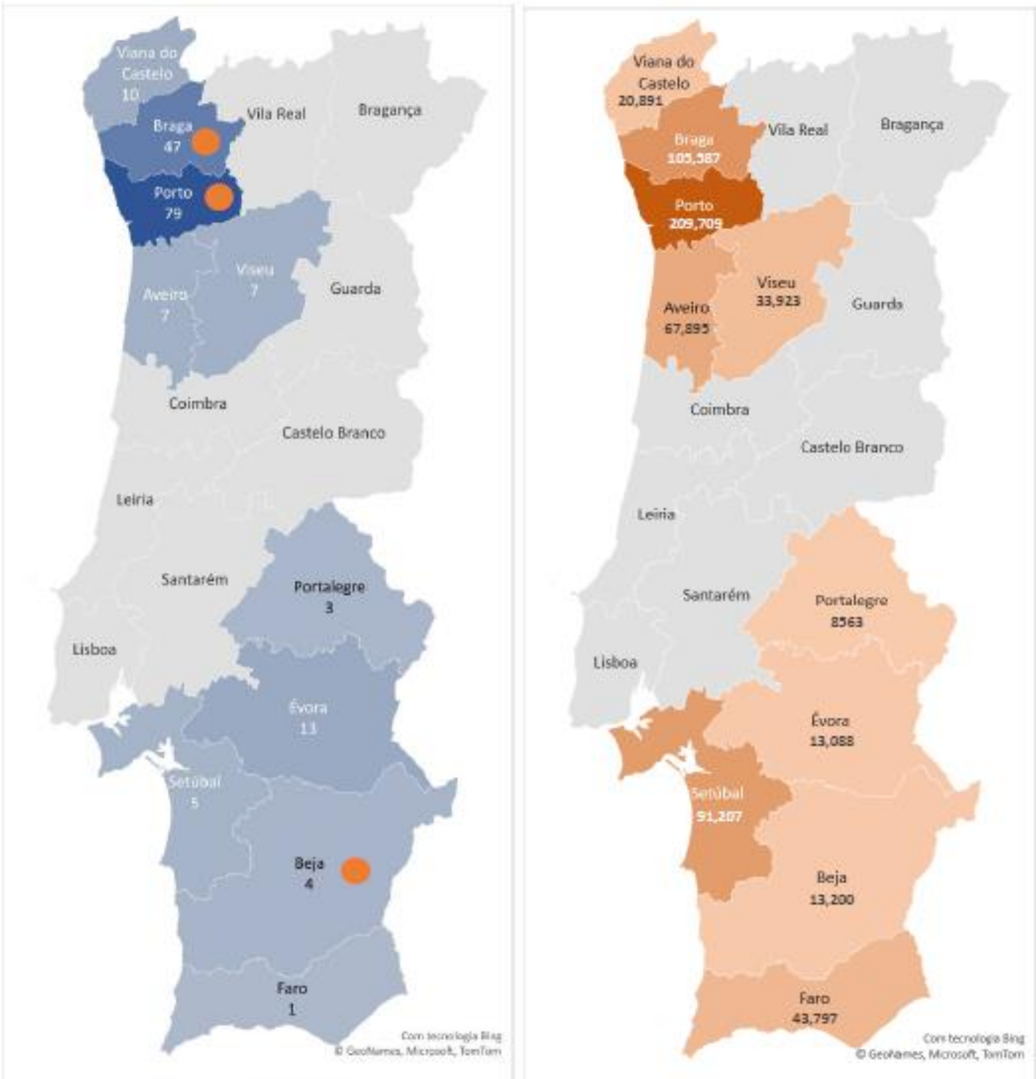
Data collected from Google Forms® and serologic analyses were downloaded in a database (Microsoft Excel 2016®; Microsoft Corp., Redmond, Washington, USA). Statistical analysis was performed with SPSS v.27.0 (IBM Corp., Armonk, New York, USA, 2020). Descriptive statistics were used to analyze data. Odds ratio was calculated to evaluate the association between cat exposition to human COVID-19 cases and the presence of antibodies against SARS-CoV-2. Homes where at least one person tested positive for SARS-CoV-2 were classified as COVID-19 positive households and those without confirmed human cases of COVID-19 were classified as COVID-19 negative households.

CHAPTER III - RESULTS

3.1 Geographic Distribution of the Sampled Cats

A total of 176 cats, belonging to 94 different households were sampled. Serum samples were obtained from 10 (of the 18) districts of mainland Portugal, although most were collected in the districts of Porto (44.9%) and Braga (26.7%) in North region of Portugal. Three out of 176 cats (1.7%) tested positive for antibodies against SARS-CoV-2, one from Porto, another from Braga and another from the district of Évora, located in the south, in Alentejo region. Most serum samples (65.0%) were obtained between June and August 2021, although the collection period was extended until the end of October 2021. Of the 10 districts investigated, there was a greater number of human COVID-19 cases in the districts of Porto and Braga at the end of serum sample collection period (Figure 5).

The number of sampled cats by household was variable, but in the most of the households (79.8%) only one cat was screened. In 10.6%, 2.1%, 2.1% and 1.1% of the households, two, three, four and five cats were sampled, respectively. In four households it was possible to collect blood from 10, 13, 16 and 21 cats during veterinary medical care provided at home.



a) b)

Figure 5- Geographical distribution of sampled cats and cumulative human COVID-19 cases. **(a)** The number of sampled cats by district is shown by the color gradient, as indicated in the legend. The district of residence of SARS-CoV-2-positive cats is marked with a circle. **(b)** The number of cumulative human COVID-19 cases at the end of October 2021 by district is shown by color gradient, as indicated in the legend.

3.2. Characterization of Households

Background information about 94 households was obtained using an online questionnaire. Most cat owners were female (86.2%), 43.6% aged between 30-50 and 29.8% between 50-70 years old. Regarding educational qualifications, 28.7% of the cat owners had a degree and 26.6% attended secondary education. As for professional activity, most cat owners were employed (73.4%) and 14.9% were retired.

Forty-seven households (50.0%) lived in Porto, 13 (13.8%) in Évora and 12 (12.8%) in Braga districts. Regarding size, investigated households were composed by 2.6 ± 1.2 (mean ± standard deviation) human members. Most households (84.0%) had other pets in addition to the sampled cat(s), namely cats (30.9%) and dogs and cats (26.6%) (Appendix III).

3.2.1. COVID-19 Positive Households

In total, 20 (21.3%) households reported at least one confirmed case of human COVID-19 and were classified as positive households. Specifically, fifteen cat owners (16.0%) were diagnosed with COVID-19, and in 18 (19.1%) households others human members were diagnosed with COVID-19. According to owners, the diagnoses of human COVID-19 cases were performed by RT-qPCR from nasopharyngeal or oropharyngeal swabs (90.0%), blood collection for serology (5.0%) or rapid antigen test from nasopharyngeal or oropharyngeal swab (5.0%). Human COVID-19 diagnoses were carried out after January 2021 (55.0%), between October-December 2020 (35.0%) and between April-September 2020 (10.0%).

COVID-19 positive households lived in Porto (45.0%), Aveiro (10.0%), Viseu (10.0%), Setúbal (10.0%), Évora (10.0%), Braga (5.0%), Beja (5.0%), and Faro (5.0%) districts. Of the 176 sampled cats, 136 (77.3%) belonged to COVID-19 negative households and 40 (22.7%) to COVID-19 positive households. The prevalence of seropositivity in cats from COVID-19 positive households was 5.0% (2/40) and from COVID-19 negative households was 0.7% (1/136), which correspond to an odds ratio of 7.2 (Figure 6).

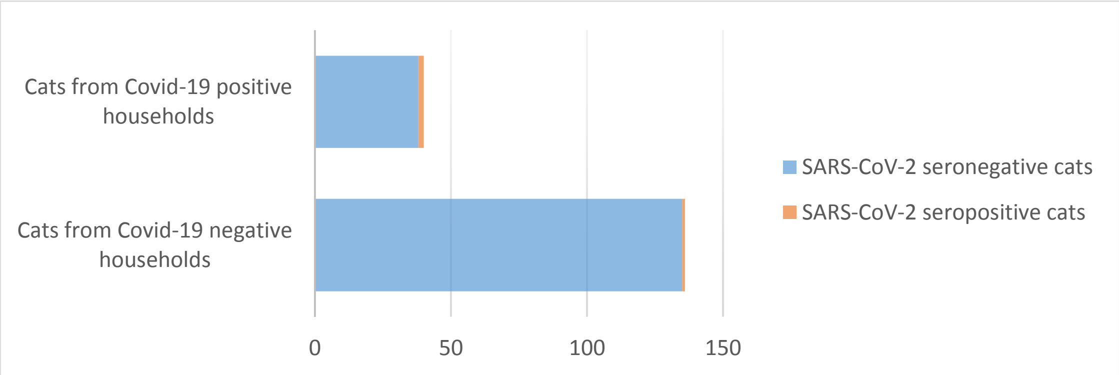


Figure 6- SARS-CoV-2 seropositivity among cats from COVID-19 positive and negative households.

3.2.2. Characterization of Cats

Background information of the 176 cats were obtained using an online questionnaire that allowed the full characterization of animals, including signalment, environment, clinical status, including the appearance of clinical signs or behavioral changes during COVID-19 pandemic and human-animal interaction before pandemic and after the diagnoses of COVID-19 human cases in the household (Appendix IV).

Most cats were female (52.8%), non-fertile (93.2%) and European Shorthaired (84.7%). Regarding to age, 45.5% of cats had between 1-5 and 39.2% between 5-10 years old. Most cats lived exclusively indoor (77.9%) and 20.5% had an indoor/outdoor lifestyle.

Concerning human-animal contact, most owners reported interacting with their cats through playing, petting, and cuddling (96.0%). Owners stated that their cat(s) rested on their lap (72.2%) and shared the bed (65.9%) and the sofa (64.8%) with them. Only one owner (0.57%) reported little interaction with her cat. Regarding frequency of interaction, most owners (48.3%) reported they interacted with the cat throughout the day and 46.0% during part of the day, for example at night. Only 6.25% of cat owners said they interacted only occasionally with the cat.

According to the owners, most cats were vaccinated annually (78.4%) and dewormed every three months (68.8%). Twenty-two cats (12.5%) had a chronic disease, namely kidney or liver disease (n=5), retroviral infection (n=5), heart disease (n=4) and gingivostomatitis (n=2), and nine of them received medication, namely food supplements for oral hygiene, feline idiopathic cystitis, behavior supplement and immunomodulators, as well as steroidal and non-steroidal anti-inflammatory drugs. According to their owners, 17.6% of cats experienced clinical or behavior changes during COVID-19 pandemic. Most reported changes were behavioral (67.7%), respiratory (6.5%) and digestive (6.5%).

3.2.3. SARS-CoV-2 Seropositive Cats

The three SARS-CoV-2 seropositive cats were neutered or sterilized, had an indoor or mixed indoor/outdoor lifestyle and cohabited with other animal species. Owners of Cat 1 and Cat 2 stated that they established close and frequent interaction with their cats and that after the diagnoses of human COVID-19 cases, household members maintained the same type and frequency of contact with cats. Owners did not notice clinical signs (respiratory, gastrointestinal, conjunctivitis or others) or behavior changes. Cat 3 was infected with Feline Immunodeficiency Virus (FIV), had a basal cell carcinoma, and received corticosteroids (Table 3).

Table 3- Characterization of SARS-CoV-2 seropositive cats.

		Cat 1	**Cat 2**	**Cat 3**
Signalment	Sex	Female	Male	Male
	Reproductive status	Neutered	Spayed	Spayed
	Age (years)	1-5	<1	>10
	Breed	European shorthair	European shorthair	Siamese
Environment	Lifestyle	Indoor	Indoor	Indoor/outdoor
	Co-habitants	Dog, cat	Dog, cat	Dog, cat, bird
Clinical status	Vaccination	Yes, annually	Yes, annually	No
	Deworming	Yes, every 3 months	Yes, every 3 months	Occasionally
	Chronic disease / treatment	No	No	Yes, FIV, basal cell carcinoma / corticosteroids
	Clinical signs or behavior changes during the pandemic	No	No	No
Interaction with humans / Frequency of interaction	Before the pandemic	Playing, petting, and cuddling Rest on the lap Sleep in bed / All day	Playing, petting, and cuddling Rest on the lap Sleep in bed / All day	Playing, petting, and cuddling Rest on the lap / During a period of the day
	After COVID-19 diagnoses	As usual	As usual	-

ELISA testing of the three SARS-CoV-2 seropositive cats presented a S/P% of 136.7343 (OD=1.7203), 242.0433 (OD=2.3851) and 145.144 (OD=1.7498). S/P% mean ± standard deviation of seropositive cats was 174.6405 ± 58.5237 (OD=1.9517 ± 0.3756), while S/P% of seronegative cats was 3.3105 ±4.8963 (OD= 0.1589 ± 0.0539). No cats tested doubtful. OD mean ± standard deviation of negative controls was 0.1400 ±0.0369 and of positive controls was 1.2010 ± 0.0994 (Figure 7).

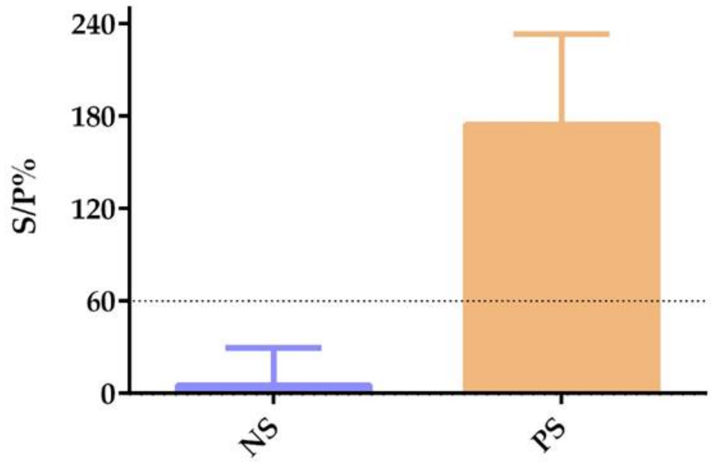


Figure 7- Anti-SARS-CoV-2 antibody screening results by ELISA. S/P% of negative samples (NS) and positive samples (PS) are represented by bar graph showing mean + standard deviation. The dotted line represents S/P% cutoff.

The owner and other human members of two SARS-CoV-2 seropositive cats (Cat 1 and Cat 2) were diagnosed COVID-19 positive by RT-qPCR between October 2020 and January 2021 (it was not possible to determine the exact date). Four cats from Cat 1 household were screened for antibodies against SARS-CoV-2, but none tested positive. Although there were other pets (dogs and cat) in Cat 2 household, only Cat 2 was tested. The COVID-19 negative household of Cat 3 had other pets (cats, dogs, and birds), but only Cat 3 was screened for anti-SARS-CoV-2 antibodies. The time interval between COVID-19 diagnoses in the household human members of Cat 1 and date of Cat 1 blood sampling was eight months. It was not possible to obtain the exact diagnose date of COVID-19 human cases of Cat 2 and Cat 3 households.

CHAPTER IV - DISCUSSION

A considerable number of studies indicated that domestic cats are highly susceptible to experimental and natural SARS-CoV-2 infection [9,10,95,96]. There are approximately 1.4 million domestic cats in Portuguese homes [97] where owners establish daily contact with them, like playing, petting, and grooming [98].

Previous studies have found a low prevalence of SARS-CoV-2 positive cats by RT-qPCR [99,100,101], but serological studies have revealed higher seroprevalences [13,102]. Thus, serological tests seem to be preferred when the objective is to determine the SARS-CoV-2 exposition in companion animals. However, as cats can be infected by FCoV, another coronavirus genetically distinct from SARS-CoV-2, the possibility of cross-reactions has been raised [102]. Nevertheless, some studies have already ruled out serological cross-reactivity of FCoV-specific antibodies with SARS-CoV-2 [18,103,104], reinforcing the usefulness of serological methods to identify exposure to SARS-CoV-2.

In this study we determined anti-SARS-CoV-2 IgG seroprevalence in a sample of 176 cats belonging to 94 households of mainland Portugal. The restrictions imposed by the pandemic, the news of euthanasia and abandonment of thousands of pets [105], the functioning of veterinary centers and the uncertainty about the role of companion animals in the spread of the virus may justify the poor adherence of veterinary centers to the study.

Published serological studies have shown distinct seropositivities in companion animals related to human infection [101,104,106,107]. In our study, the overall seroprevalence was 1.7%. At the end of October 2021, when the collection of serum samples ended, the cumulative cases of human COVID-19 in Portugal was 1,09 million. Seropositivity found in this study is slightly higher than the seroprevalence found in the first large scale survey conducted in Germany from April to September 2020, a period when the incidence of human COVID-19 in the country was still rather low [104]. However, the seroprevalence obtained in the present work was much lower compared with the seroprevalence obtained in another Portuguese study (21.7%) [13] that collected samples from 69 cats between December 2020 and May 2021 in the districts of Braga, Porto, and Lisboa. The differences may be related to sampling method, period of serum sample collection, geographical distribution of sampled cats and the use of distinct ELISA assays with different specificities and sensitivities in the two studies.

In this study, of the 10 districts surveyed, there was a greater number of human COVID-19 cases in the districts of Porto and Braga at the end of serum sample collection period. Not surprisingly, the three SARS-CoV-2 seropositive cats were identified in these three districts.

Considering COVID-19 status, our study included 20 negative and 76 positive households. Of the three seropositive cats identified in this study, two belonged to COVID-19 positive households and one cat lived in a COVID-19 negative household. Thus, the seropositivity observed in cats from COVID-19 positive households was 5.0% and in cats from COVID-19 negative households was 0.7%. Several studies have already reported the infection of cats following natural exposure to infected people [103,108]. In line with this, high seropositivities were observed in cats living in COVID-19 positive households [8,99,109]. As such we hypothesize that the most likely source of infection of the two seropositive cats from COVID-19 positive households identified in this study was the contact with human infected members ("reverse zoonosis"). Indeed, both cats had an exclusively indoor lifestyle, which limits the possibility of infection in another environment.

However, the source of infection of the cat with mixed indoor/outdoor lifestyle belonging to the COVID-19 negative household is more difficult to identify but there are the possibilities of infection via SARS-CoV-2 environmental contamination or cat-to-cat transmission [110,111,112]. Furthermore, cat-to-cat transmission in natural conditions is consistent with experimental studies that proved virus transmission from infected to naive cats via the airborne route [8,9,95,113]. Meanwhile, other studies had already reported the presence of seropositive cats with outdoor access living in COVID-19 negative households [13,113].

Determination of antibody longevity is crucial to evaluate humoral response to SARS-CoV-2 under natural conditions. Experimental studies determined that cats seroconverted 7 to 14 days post infection [9], however the duration of antibodies is unknown. In the present study one cat tested positive eight months after the diagnoses of COVID-19 in human household members, suggesting longer antibody longevity.

One of three seropositive cats identified in this study was co-infected with FIV. Viral co-infection was already reported in other studies [13,111] and may play an important role in the susceptibility and clinical outcome of SARS-CoV-2 infected cats [13]. However, in this study all SARS-CoV-2 seropositive cats, including the FIV-co-infected, were apparently asymptomatic, as their owners did not notice any clinical sign or behavioral change. Probably, the nonspecific, mild, and reversible character of the clinical signs associated with COVID-19 in companion animals [108] prevented the recognition of the disease by cat owners or it can be speculated that the chronic administration of corticosteroids to this cat, due to a neoplasm, may have contributed to decreasing the inflammation associated with SARS-CoV-2 infection and the manifestation of clinical signs, although the beneficial effect of this drug in humans with COVID-19 is controversial [114].

CHAPTER V - CONCLUSION AND CHALLENGES

The emergence of the SARS-CoV-2 virus had a great worldwide impact, since there was no knowledge of its etiology and epidemiology, requiring quick and assertive methods to identify alternatives for treatment and improve prevention measures. Since pets are becoming more and more an integral part of most families all over the world and large and wild animals are often our work base, emerge the importance of studying the ability of infection of this recent virus in animals.

During the review and research work for the present dissertation, it was possible to stablish that the coexistence between humans and animals is crucial for the spread of the virus, being the transmission from humans to animals the most important route in the transmission chains.

It is still premature to estimate the effects of SARS-CoV2 on the above-mentioned target species, especially in co-infection with viruses shared among the species, while the severity of the health status of co-infected cats, especially FIP, FIV and FeLV, is already known. In order to stop the transmission of this virus, it is clear the requisition of preventive measures, such as washing and disinfecting hands before and after handling the animal, reducing cohabitation and/or wearing mask and gloves and keeping the cats indoors during quarantine while the owner/handler is infected.

Also, the authors of the present work advocates inspection and regulation of services by veterinary authorities to ensure reduction of virus transmission and animal welfare. However, this study highlights the low risk of SARS-CoV-2 transmission from COVID-19 positive human household members to domestic cats, even in a context of close and frequent human-animal contact.

REFERENCES

1. Decaro, N.; Martella, V.; Saif, L.J.; Buonavoglia, C. COVID-19 from veterinary medicine and one health perspectives: What animal coronaviruses have taught us. *Research in Veterinary Science* **2020**, *131*, 21-23, doi: 10.1016/j.rvsc.2020.04.009.

2. Ma, L.; Song, K.; Huang, Y. Coronavirus Disease-2019 (COVID-19) and Cardiovascular Complications. *Journal of Cardiothoracic and Vascular Anesthesia* **2021**, *35*, 1860-1865, doi: 10.1053/j.jvca.2020.04.041.

3. Valencak, T.G.; Csiszar, A.; Szalai, G.; Podlutsky, A.; Tarantini, S.; Fazekas-Pongor, V.; Papp, M.; Ungvari, Z. Animal reservoirs of SARS-CoV-2: calculable COVID-19 risk for older adults from animal to human transmission. *GeroScience* **2021**, *43*, 2305–2320, doi: 10.1007/s11357-021-00444-9.

4. Kannan, S.; Ali, P.S.S.; Sheeza, A.; Hemalatha, K. COVID-19 (Novel Coronavirus 2019) – recent trends. *European Review for Medical and Pharmacological Sciences* **2020**, *24*, 2006-2011, doi: 10.26355/eurrev_202002_20378.

5. Prata, J.C. Strategies for the Improvement of Pet Health and Welfare in Portugal Based on a Pilot Survey on Husbandry, Opinion, and Information Needs. *Animals* **2020**, *10*, 848. doi: 10.3390/ani10050848.

6. Overgaauw, P.A.M.; Vinke, C.M. A One Health Perspective on the Human-Companion Animal Relationship with Emphasis on Zoonotic Aspects. *Int J Environ Res Public Health* **2020**, *17*, 3789. doi: 10.3390/ijerph17113789.

7. Hobbs, E.C.; Reid, T.J. Animals and SARS-CoV-2: Species susceptibility and viral transmission in experimental and natural conditions, and the potential implications for community transmission. *Transbound Emerg Dis.* **2021**, *68*, 1850–1867, doi: 10.1111/tbed.13885.

8. Halfmann, P.J.; Hatta, M.; Chiba, S.; Maemura, T.; Fan, S.; Takeda, M.; Kinoshita, N.; Hattori, S-I.; Sakai-Tagawa, Y.; Iwatsuki-Horimoto, K.; et al. Transmission of SARS-CoV-2 in Domestic Cats. *N Engl J Med* **2020**, *383*, 592-594, doi: 10.1056/NEJMc2013400.

9. Shi, J.; Wen, Z.; Zhong, G.; Yang, H.; Wang, C.; Huang, B.; Liu, R.; He, X.; Shuai, L.; Sun, H.; et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science* **2020**, *368*, 1-5, doi: 10.1126/science.abb7015.

10. Barrs, V.R.; Peiris, M.; Tam, K.W.S.; Law, P.Y.T.; Brackman, C.J.; To, E.M.W.; Yu, V.Y.T.; Chu, D.K.W.; Perera, R.A.P.M.; Sit, T.H.C. SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. *Emerging Infectious Diseases* **2020**, *26*, 3071–3074, doi: 10.3201/eid2612.202786.

11. Garigliany, M.; Van Laere, A.-S.; Clercx, C.; Giet, D.; Escriou, N.; Huon, C.; van der Werf, S.; Eloit, M.; Desmecht, D. SARS-CoV-2 Natural Transmission from Human to

Cat, Belgium, March 2020. *Emerg Infect Dis* **2020**, *26*, 3069–3071, doi: 10.3201/eid2612.202223.

12. Temmam, S.; Barbarino, A.; Maso, D.; Behillil, S.; Enouf, V.; Huon, C.; Jaraud, A.; Chevallier, L.; Backovic, M.; Pérot, P.; et al. Absence of SARS-CoV-2 infection in cats and dogs in close contact with a cluster of COVID-19 patients in a veterinary campus. *One Health* **2020**, *10*, 1-4, doi: 10.1016/j.onehlt.2020.100164.

13. Barroso, R.; Vieira-Pires, A.; Antunes, A; Fidalgo-Carvalho, I. Susceptibility of Pets to SARS-CoV-2 Infection: Lessons from a Seroepidemiologic Survey of Cats and Dogs in Portugal. *Microorganisms* **2022**, *10*, 1-14, doi: 10.3390/microorganisms10020345.

14. Teixeira, A.I.P. SARS-CoV-2 in animals: what about the cat?. *Veterinary Quarterly* **2021**, *41*, 226-227, doi: 10.1080/01652176.2021.1958393.

15. Clayton, E,; Ackerley, J.; Aelmans, M.; Ali, N.; Ashcroft, Z.; Ashton, C.; Barker, R.; Budryte, V.; Burrows, C.; Cai, S.; et al. Structural Bases of Zoonotic and Zooanthroponotic Transmission of SARS-CoV-2. *Viruses* **2022**, *14*, 1-22, doi: 10.3390/v14020418.

16. Bakhiet, M.; Taurin, S. SARS-CoV-2: Targeted managements and vaccine development. *Cytokine and Growth Factor Reviews* **2021**, *58*, 16–29, doi: 10.1016/j.cytogfr.2020.11.001.

17. Kim, Y.I.; Casel, M.A.B.; Choi, Y.K. Transmissibility and pathogenicity of SARS-CoV-2 variants in animal models. *Journal of Microbiology* **2022**, *60*, 255–267, doi: 10.1007/s12275-022-2033-z.

18. Fritz, M.; Rosolen, B.; Krafft, E.; Becquart, P.; Elguero, E.; Vratskikh, O.; Denolly, S.; Boson, B.; Vanhomwegen, J.; Gouilh, M.A. High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. *One Health* **2020**, *11*, 1-5, doi: 10.1016/j.onehlt.2020.100192.

19. de Vries, R.D.; Rockx, B.; Haagmans, B.L.; Herfst, S.; Koopmans, M.P.G.; de Swart, R.L. Animal models of SARS-CoV-2 transmission. *Current Opinion in Virology* **2021**, *50*, 8-16, doi: 10.1016/j.coviro.2021.06.007.

20. Hillen, H.S. Structure and function of SARS-CoV-2 polymerase. *Current Opinion in Virology* **2021**, *48*, 82–90, doi: 10.1016/j.coviro.2021.03.010.

21. Leroy, E.M.; Gouilhb, M.A.; Brugère-Picouxc, J. The risk of SARS-CoV-2 transmission to pets and other wild and domestic animals strongly mandates a one-health strategy to control the COVID-19 pandemic. *One Health* **2020**, *10*, 1-4, doi: 10.1016/j.onehlt.2020.100133.

22. Brant, A.C.; Tien, W.; Majerciak, V.; Yang, W.; Zheng, Z-M. SARS-CoV-2: from its discovery to genome structure, transcription, and replication. *Cell & Bioscience* **2021**, *11*, 1-17, doi: 10.1186/s13578-021-00643-z.

23. Yang, H.; Rao, Z. Structural biology of SARS- CoV-2 and implications for therapeutic development. *Nature Reviews* **2021**, *19*, 685-700, doi: 10.1038/s41579-021-00630-8.

24. Ferreira, G.; Santander, A.; Savio, F.; Guirado, M.; Sobrevia, L.; Nicolson, G.L. SARS-CoV-2, Zika viruses and mycoplasma: Structure, pathogenesis and some treatment options in these emerging viral and bacterial infectious diseases. *BBA - Molecular Basis of Disease* **2011**, *1867*, 1-30, doi: 10.1016/j.bbadis.2021.166264.

25. Goraichuk, I.V.; Arefiev, V.; Stegniy, B.T.; Gerilovych, A.P. Zoonotic and Reverse Zoonotic Transmissibility of SARS-CoV-2. *Virus Research* **2021**, *302*, 1-15, doi: 10.1016/j.virusres.2021.198473.

26. Segars, J.; Katler, Q.; McQueen, D.B.; Kotlyar, A.; Glenn, T.; Knight, Z.; Feinberg, E.C.; Taylor, H.S.; Toner, J.P.; Kawwass, J.F. Prior and novel coronaviruses, Coronavirus Disease 2019 (COVID-19), and human reproduction: what is known? *Fertility and Sterility* **2020**, *113*, 1140-1149, doi: 10.1016/j.fertnstert.2020.04.025.

27. Jaimes, J.A.; Whittaker, G.R. Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function. *Virology* **2018**, *517*, 108–121, doi: 10.1016/j.virol.2017.12.027.

28. Liu, Q.; Gerdts, V. Transmissible Gastroenteritis Virus of Pigs and Porcine Epidemic Diarrhea Virus (Coronaviridae). *Encyclopedia of Virology* **2021**, *2*, 850-853, doi: 10.1016/B978-0-12-809633-8.20928-X.

29. Vlasova, A. N.; Wang, Q.; Jung, K.; Langel, S. N.; Malik, Y.S.; Saif, L. J. Porcine Coronaviruses. *Emerging and Transboundary Animal Viruses* **2020**, 79-110, doi: 10.1007/978-981-15-0402-0_4.

30. Tian, Z.; Pan, Q.; Zheng, M.; Deng, Y.; Guo, P.; Cong, F.; Hu, X. Molecular characterization of the FCoV-like canine coronavirus HLJ-071 in China. *BMC Veterinary Research* **2021**, *17*, 1-9, doi: 10.1186/s12917-021-03073-8.

31. Wang, M-Y.; Zhao, R.; Gao, L-J.; Gao, X-F.; Wang, D-P.; Cao, J-M. SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. *Frontiers in Cellular and Infection Microbiology* **2020**, *10*, 1-17, doi: 10.3389/fcimb.2020.587269.

32. Latinne, A.; Hu, B.; Olival, K.J.; Zhu, G.; Zhang, L.; Li, H.; Chmura, A.A.; Field, H.E.; Zambrana-Torrelio, C.; Epstein, J.H.; et al. Origin and cross-species transmission of bat coronaviruses in China. *Nature Communications* **2020**, *11*, 1-15, doi: 10.1038/s41467-020-17687-3.

33. Ellis, J. What is the evidence that bovine coronavirus is a biologically significant respiratory pathogen in cattle?. *The Canadian Veterinary Journal* **2019**, *60*, 147–152.

34. Erles, K.; Brownlie, J. Canine Respiratory Coronavirus: An Emerging Pathogen in the Canine Infectious Respiratory Disease Complex. *Vet Clin Small Anim* **2008**, *38*, 815-825, doi: 1016/j.cvsm.2008.02.008.

35. Miszczak, F.; Tesson, V.; Kin, N.; Dina, J.; Balasuriya, U.B.R.; Pronost, S.; Vabret, A. First detection of equine coronavirus (ECoV) in Europe. *Veterinary Microbiology* **2014**, *171*, 206-209, doi: 10.1016/j.vetmic.2014.03.031.

36. Hornberger, F.R. Enterotropic mouse hepatitis virus. *Laboratory Animals* **1997**, *31*, 97-115, doi: 10.1258/002367797780600189.

37. Omrani, A.S.; Al-Tawfiq, J.A.; Memish, Z.A. Middle East respiratory syndrome coronavirus (MERS-CoV): animal to human interaction. *Pathogens and Global Health* **2015**, *109*, 354-362, doi: 10.1080/20477724.2015.1122852.

38. Du, L.; He, Y.; Zhou, Y.; Liu, S.; Zheng, B-J.; Jiang, S. The spike protein of SARS-CoV — a target for vaccine and therapeutic development. *NATURE REVIEWS* **2009**, *7*, 226-236, doi: 1038/nrmicro2090.

39. World Animal Health Information System (WOAH). Avian Infectious Bronchitis. WOAH Terrestrial Manual 2018, chapter 2.3.2., 1-14.

40. Guy, J.S. Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronaviruses: A review. *Avian Pathology* **2000**, *29*, 207– 212, doi: 10.1080/03079450050045459.

41. Sallard, E.; Halloy, J.; Casane, D.; Decroly, E.; van Helden, J. Tracing the origins of SARS-COV-2 in coronavirus phylogenies: a review. *Environmental Chemistry Letters* **2021**, 1-17, doi: 10.1007/s10311-020-01151-1.

42. Rahimi, P.; Islam, M.S.; Duarte, P.M.; Tazerji, S.S.; Sobur, M.A.; El Zowalaty, M.E.; Ashour, H.M.; Rahman, M.T. Impact of the COVID-19 pandemic on food production and animal health. *Trends in Food Science & Technology* **2022**, *121*, 105-113, doi: 10.1016/j.tifs.2021.12.003.

43. Lin, C-N.; Chan, K.R.; Ooi, E.E.; Chiou, M-T.; Hoang, M.; Hsueh, P-R.; Ooi, P.T. Animal Coronavirus Diseases: Parallels with COVID-19 in Humans. *Viruses* **2021**, *13*, 1-15, doi: 10.3390/v13081507.

44. Bonilauri, P.; Rugna, G. Animal Coronaviruses and SARS-COV-2 in Animals, What Do We Actually Know?. *Life* **2021**, *11*, 1-17, doi: 10.3390/life11020123.

45. Meekins, D.A.; Gaudreault, N.N.; Richt, J.A. Natural and Experimental SARS-CoV-2 Infection in Domestic and Wild Animals. *Viruses* **2021**, *13*, 1-44, doi: 10.3390/v13101993.

46. Khbou, M.K.; Jedidi, M.D.; Zaafouri, F.B.; Benzarti, M. Coronaviruses in farm animals: Epidemiology and public health implications. *Vet. Med. Sci.* **2021**, *7*, 322–347, doi: 10.1002/vms3.359.

47. Duarte, M.; Nogueira, T.; Botelho, A.; Cavaco, S.; Duarte, A.; Santos, P.T.; de Sousa, R.; Portela, S. Evolução e Patogenia do Vírus e Prevenção da Doença. In Coronavírus dos

animais e do Homem, INIAV - Instituto Nacional de Investigação Agrária e Veterinária, I.P. República Portuguesa, 2020, 1-50, ISBN 978-972-579-055-7.

48. World Small Animal Veterinary Association. Conselhos Para Veterinários Sobre Vacinação De Rotina Durante A Pandemia De COVID-19. Available online: https://wsava.org/global-guidelines/vaccination-guidelines/ (accessed on 24 October 2020).

49. Stout, A.E.; André, N.M.; Jaimes, J.A.; Millet, J.K.; Whittaker, G.R. Coronaviruses in cats and other companion animals: Where does SARS-CoV-2/COVID-19 fit? *Veterinary Microbiology* **2020**, *247*, 1-7, doi: 10.1016/j.vetmic.2020.108777.

50. Decaro, N; Lorusso, A. Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. *Veterinary Microbiology* **2020**, *244*, 1–18, doi: 10.1016/j.vetmic.2020.108693.

51. World Organisation for Animal Health. (WOAH). Questions and answers on the COVID-19 (em linha). Available online: https://www.oie.int/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/ (accessed on 30 May, 2020).

52. Klaus J, Meli ML, Willi B, Nadeau, S.; Beisel, C.; Stadler, T.; Egberink, H.; Zhao, S.; Lutz, H.; Riond, B.; et al. Detection and Genome Sequencing of SARS-CoV-2 in a Domestic Cat with Respiratory Signs in Switzerland. *Viruses* **2021**, *13*, 1-28, doi: 10.3390/v13030496.

53. Jemerši´c, L.; Lojki´c, I.; Kreši´c, N.; Keros, T.; Zelenika, T.A.; Jurinovi´c, L.; Skok, D.; Bata, I.; Boras, J.; Habrun, B.; et al. Investigating the Presence of SARS CoV-2 in Free-Living and Captive Animals. *Pathogens* **2021**, *10*, 1-12, doi: 10.3390/pathogens10060635.

54. Zhang, J.; Xiao, T.; Cai, Y.; Chen, B. Structure of SARS-CoV-2 spike protein. *Current Opinion in Virology* **2021**, *50*, 173-182, doi: 10.1016/j.coviro.2021.08.010.

55. Hassan, S.S; Lundstrom, K; Serrano-Aroca, A; Adadi, P.; Aljabali, A.A.A.; Redwan, E.M.; Lal, A.; Kandimalla, R.; El-Aziz, T.M.A.; Choudhury, P.P.; Azad, G.K; et al. Emergence of unique SARS-CoV-2 ORF10 variants and their impact on protein structure and function. *International Journal of Biological Macromolecules* **2022**, *194*, 128-143, doi: 10.1016/j.ijbiomac.2021.11.151.

56. Giovanetti, M.; Benedetti, F.; Campisi, G.; Ciccozzi, A.; Fabris, S.; Ceccarelli, G.; Tambone, V.; Caruso, A.; Angeletti, S.; Zella, D; et al. Evolution patterns of SARS-CoV-2: Snapshot on its genome variants. *Biochemical and Biophysical Research Communications* **2021**, *538*, 88-91, doi: 10.1016/j.bbrc.2020.10.102.

57. Harrison, A.G; Lin, T.; Wang, P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends in Immunology* **2020**, *41*, 1100-1115, doi: 0.1016/j.it.2020.10.004.

58. Singhal, T. A review of coronavirus disease-2019 (COVID-19). *The Indian Journal of Pediatrics*, **2020**, *87*, 281–286, doi:10.1007/s12098-020-03263-6.

59. Do Vale B, Lopes AP, Fontes MDC, Silvestre, M.; Cardoso, L.; Coelho, A.C. Bats, pangolins, minks and other animals - villains or victims of SARS-CoV-2? *Veterinary Research Communications* **2021**, *19*, 1–19, doi: 10.1007/s11259-021-09787-2.

60. Sharun, K.; Dhama, K.; Pawde, A.M.; Gortázar, C.; Tiwari, R.; Bonilla-Aldana, D.K.; Rodriguez-Morales, A.J.; de la Fuente, J.; Michalak, I.; Attia, Y.A. SARS-CoV-2 in animals: potential for unknown reservoir hosts and public health implications. *Veterinary Quarterly* **2021**, *41*, 181-201, doi: 10.1080/01652176.2021.1921311.

61. Hosie, M.J; Hofmann-Lehmann, R.; Hartmann, K.; Egberink, H.; Truyen, U.; Addie, D.D.; Belák, S.; Boucraut-Baralon, C.; Frymus, T.; Lloret, A.; et al. Anthropogenic Infection of Cats during the 2020 COVID-19 Pandemic. *Viruses* **2021**, *13*, 1-13, doi: 10.3390/v13020185.

62. Cleary, S.J.; Pitchford, S.C., Amison, R.T., Carrington, R., Cabrera, C.L.R.; Magnen, M.; Looney, M.R.; Gray, E.; Page, C.P. Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. *Br J Pharmacol* **2020**, *177*, 4851–4865, doi: 10.1111/bph.15143.

63. Logeot, M.; Mauroy, A.; Thiry, E.; De Regge, N.; Vervaeke, M.; Beck, O.; De Waele, V.; Van den Berg, T. Risk assessment of SARS-CoV-2 infection in free-ranging wild animals in Belgium. *Transbound Emerg Dis*. **2021**; *00*, 1-11, doi: 10.1111/tbed.14131.

64. Saravanan, U.B.; Namachivayam, M.; Jeewon, R.; Huang, J.D.; Durairajan, S.S.K. Animal models for SARS-CoV-2 and SARS-CoV-1 pathogenesis, transmission and therapeutic evaluation. *World J Virol* **2022**, *11*, 40-56, doi: 10.5501/wjv.v11.i1.40.

65. Korath, A.D.J.; Janda, J.; Untersmayr, E.; Sokolowska, E.; Feleszko, W.; Agache, J.; Seida, A.A.; Hartmann, K.; Jensen-Jarolim, E.; Pali-Schöll, I. One Health: EAACI Position Paper on coronaviruses at the human-animal interface, with a specific focus on comparative and zoonotic aspects of SARS-Cov-2. *Allergy* **2021**, *00*, 1-17, doi: 10.1111/all.14991.

66. Kiros, M.; Andualem, H.; Kiros, T.; Hailemichael, W.; Getu, S.; Geteneh, A.; Alemu, D.; Abegaz, W.E. COVID-19 pandemic: current knowledge about the role of pets and other animals in disease transmission. *Virology Journal* **2020**; *17*, 1-8, doi: 10.1186/s12985-020-01416-9.

67. Maurin, M.; Fenollar, F.; Mediannikov, O.; Davoust, B.; Devaux, C.; Raoult, D. Current Status of Putative Animal Sources of SARS-CoV-2 Infection in Humans: Wildlife, Domestic Animals and Pets. *Microorganisms* **2021**, *9*, 1-29, doi: 10.3390/microorganisms9040868.

68. AFCD. Agriculture, Fisheries and Conservation Department. Detection of low level of COVID-19 virus in pet dog. Available online:

https://www.info.gov.hk/gia/general/202002/28/P2020022800013.htm (accessed on 10 May, 2022).

69. AFCD. Agriculture, Fisheries and Conservation Department. Pet dog tests positive for COVID-19 virus. Available online: https://www.info.gov.hk/gia/general/202003/19/P2020031900606.htm (accessed on 10 May, 2022).

70. David, G. Quarantine the cat? Disinfect the dog? The latest advice about the coronavirus and your pets. Available online: https://www.science.org/content/article/quarantine-cat-disinfect-dog-latest-advice-about-coronavirus-and-your-pets (accessed on Abril 30, 2022).

71. Goumenou, M.; Spandidos, D.; Tsatsakis, A. Possibility of transmission through dogs being a contributing factor to the extreme Covid-19 outbreak in North Italy. Molecular *Medicine Reports* **2020**, *21*, 2293-2295, doi: 10.3892/mmr.2020.11037.

72. Sit, T.H.C.; Brackman, C.J.; Ip, S.M.; Tam, K.W.S.; Law, P.Y.T.; To, E.M.W.; Yu, V.Y.T.; Sims, L.D.; Tsang, D.N.C.; Chu, D.K.W.; et al. Infection of Dogs with SARS-CoV-2. *Nature* **2020**, *586*, 776–778, doi: 10.1038/s41586-020-2334-5.

73. Thompson H. A cat appears to have caught the coronavirus, but it’s complicated. Available online: https://www.sciencenews.org/article/cats-animals-pets-coronavirus-covid19 (accessed on 20 April, 2022).

74. AFCD. Agriculture, Fisheries and Conservation Department. Pet cat tests positive for COVID-19 virus. Available online: https://www. info.gov.hk/gia/general/202003/31/P2020033100717.htm (accessed on 10 May, 2022).

75. AVMA. American Veterinary Medical Association. SARS-CoV-2 in animals, including pets. Available online: https://www.avma.org/resources-tools/one-health/covid-19/sars-cov-2-animals-including-pets (accessed on 10 May, 2022).

76. United States Department of Agriculture. Confirmed cases of SARS-CoV-2 in animals in the United States (USDA). Available online: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/SA_One_Health/sars-cov-2-animals-us (accessed on 30 April, 2022).

77. Tiwari, R.; Dhama, K.; Sharun, K.; Yatoo, M.I.; Malik, Y.S.; Singh, R.; Michalak, I.; Sah, R.; Bonilla-Aldana, D.K.; Rodriguez-Morales, A.J. COVID-19: animals, veterinary and zoonotic links. *Veterinary Quarterly* **2020**, *40*, 169-182, doi: 10.1080/01652176.2020.1766725.

78. Farag, E.A; Islam, Md.M; Enan, K; El-Hussein, A-R.M; Bansal, D.; Haroun, M. SARS-CoV-2 at the human-animal interface: A review. *Heliyon* **2021**, *7*, 2405-8440, doi: 10.1016/j.heliyon.2021.e08496.

79. Shriner SA, Ellis JW, Root JJ, Roug A, Stopak SR, Wiscomb GW, Zierenberg JR, Ip HS, Torchetti MK and DeLiberto TJ. SARS-CoV-2 Exposure in Escaped Mink, Utah, USA. *Emerging Infectious Diseases* **2021**, *27*, 988-990, doi: 10.3201/eid2703.204729.

80. Ganesh, B.; Rajakumar, T.; Malathi, M.; Manikandan, N.; Nagaraj, J.; Santhakumar, A.; Elangovan, A.; Malik, Y.S. Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives. *Clinical Epidemiology and Global Health* **2021**, *10*, 1-10, doi: 10.1016/j.cegh.2020.100694.

81. Parry, N.M.A. COVID-19 and pets: When pandemic meets panic. *Forensic Science International* **2020**, *2*, 1-4, doi: 10.1016/j.fsir.2020.100090.

82. Shende P, Gaikwad P, Gandhewar M, Ukey P, Bhide A, Patel V, Bhagat S, Bhor V, Mahale S, Gajbhiye R and Modi D. Persistence of SARS-CoV-2 in the first trimester placenta leading to transplacental transmission and fetal demise from an asymptomatic mother. *European Society of Human Reproduction and Embryology* **2020**, *36*, 1-26, doi: 10.1093/humrep/deaa367.

83. Delahay RJ, de la Fuente, J.; Smith, G.C.; Sharun, K.; Snary, E.L.; Girón, L.F.; Nziza, J.;Fooks, A.R.; Brookes, S.M.; Lean, F.Z.X.; et al. Assessing the risks of SARS-CoV-2 in wildlife. *One Health Outlook* **2021**, *3*, 1-14, doi: 10.1186/s42522-021-00039-6.

84. Koeppel, K.N.; Mendes, A.; Strydom, A.; Rotherham, L.; Mulumba, M.; Venter, M. SARS-CoV-2 Reverse Zoonoses to Pumas and Lions, South Africa. *Viruses* **2022**; 14: 1-12, doi: 10.3390/v14010120.

85. Prince, T.; Smith, S.L.; Radford, A.D.; Solomon, T.; Hughes, G.L.; Patterson, E.I. SARS-CoV-2 Infections in Animals: Reservoirs for Reverse Zoonosis and Models for Study. *Viruses* **2021**, *13*, 1-14, doi: 10.3390/v13030494.

86. Zambrano-Mila, M.S.; Freire-Paspuel, B.; Orlando, S.A.; Garcia-Bereguiain, M.A. SARS-CoV-2 infection in free roaming dogs from the Amazonian jungle. *One Health* **2022**, *14*, 1-4, doi: 10.1016/j.onehlt.2022.100387.

87. Murphy, H.L.; Ly, H. Understanding the prevalence of SARS-CoV-2 (COVID-19) exposure in companion, captive, wild, and farmed animals. *Virulence* **2021**, *12*, 2777–2786, doi: 10.1080/21505594.2021.1996519.

88. Dhama, K.; Patel, S.K.; Sharun, K.; Pathak, M.; Tiwari, R.; Yatoo, M.I.; Malik, Y.S.; Sah, R.; Rabaan, A.A.; Panwar, P.K.; et al. SARS-CoV-2 jumping the species barrier: Zoonotic lessons from SARS, MERS and recent advances to combat this pandemic virus. *Travel Medicine and Infectious Disease* **2020**, *37*, 1-12, doi: 10.1016/j.tmaid.2020.101830

89. Shehata, A.A.; Attia, Y.A.; Rahman, M.T.; Basiouni, S.; El-Seedi, H.R.; Azhar, E.I.; Khafaga, A.F.; Hafez, H.M. Diversity of Coronaviruses with Particular Attention to the Interspecies Transmission of SARS-CoV-2. *Animals* **2022**, *12*, 1-21, doi: 10.3390/ani12030378.

90. CFDCP. Centers For Disease Control and Prevention. Available online: https://www.cdc.gov/coronavirus/2019-ncov/animals/animal-testing.html (accessed on May 15, 2020).

91. Priestnall, S.L. Canine Respiratory Coronavirus: A Naturally Occurring Model of COVID-19?. *Veterinary Pathology* **2020**, *57*, 467-471, doi: 10.1177/0300985820926485.

92. Sellars, L.; Bernotas, K.; Sebo, Jeff. One Health, COVID-19, and a Right to Health for Human and Nonhuman Animals. *Health and Human Rights Journal* **2021**, *23*, 35-47.

93. Sironi, V.A.; Inglese, S.; Lavazza, A. The "One Health" approach in the face of Covid-19: how radical should it be?. *Philosophy, Ethics, and Humanities in Medicine* **2022**, *17*, 1-10, doi: 10.1186/s13010-022-00116-2.

94. Jørgensen, H.J.; das Neves, C. COVID-19: One world, one health. *Tidsskrift for Den norske legeforening* **2020**, *15*, 1-3, doi: 10.4045/tidsskr.20.0212.

95. Gaudreault, N.N.; Trujillo, J.D. SARS-CoV-2 infection, disease and transmission in domestic cats. *Emerg Microbes Infect*. **2020**, *9*, 2322-2332. doi: 10.1080/22221751.2020.1833687.

96. Hamer, S.A.; Pauvolid-Corrêa, A. SARS-CoV-2 Infections and Viral Isolations among Serially Tested Cats and Dogs in Households with Infected Owners in Texas, USA. *Viruses* **2021**, *13*, 938. doi: 10.3390/v13050938.

97. GfKTrack. 2PETs Portugal é um País Pet-Friendly. [Portugal is Pet Friendly Country]. Available online: https://www.gfk.com/pt/insights/press-release/portugal-e-um-pais-pet-friendly/ (accessed on 1 April 2022).

98. Alho, A.M.; Pontes, J. Guardians' Knowledge and Husbandry Practices of Feline Environmental Enrichment. *J Appl Anim Welf Sci*. **2016**, *19*, 115-125. doi: 10.1080/10888705.2015.1117976.

99. Patterson, E.I.; Elia, G, Grassi A. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Preprint. *bioRxiv*. **2020**, 2020.07.21.214346. doi:10.1101/2020.07.21.214346.

100. Michael, H.T.; Waterhouse, T. Frequency of respiratory pathogens and SARS-CoV-2 in canine and feline samples submitted for respiratory testing in early 2020. *J Small Anim Pract*. **2021**, *62*, 336-342. doi: 10.1111/jsap.13300.

101. Klaus, J.; Zini, E. SARS-CoV-2 Infection in Dogs and Cats from Southern Germany and Northern Italy during the First Wave of the COVID-19 Pandemic. *Viruses* **2021**, *13*, 1453. doi: 10.3390/v13081453.

102. Yilmaz, A.; Kayar, A. Presence of Antibodies to SARS-CoV-2 in Domestic Cats in Istanbul, Turkey, Before and After COVID-19 Pandemic. *Front Vet Sci*. **2021**, 8:707368. doi: 10.3389/fvets.2021.707368.

103. Zhang, Q.; Zhang, H. A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg. *Microbes Infect.* **2020**, *9*, 2013–2019. doi: 10.1080/22221751.2020.1817796.

104. Michelitsch, A.; Hoffmann, D. Occurrence of Antibodies against SARS-CoV-2 in the Domestic Cat Population of Germany. *Vaccines (Basel).* **2020**, *8*, 772. doi: 10.3390/vaccines8040772.

105. McNamara, T.; Richt, J.A. A Critical Needs Assessment for Research in Companion Animals and Livestock Following the Pandemic of COVID-19 in Humans. *Vector Borne Zoonotic Dis*. **2020**, *20*, 393-405. doi: 10.1089/vbz.2020.2650.

106. Dileepan, M.; Di, D. Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. *Virulence.* **2021**, *12*, 1597-1609. doi: 10.1080/21505594.2021.1936433.

107. Schulz, C.; Martina, B. SARS-CoV-2-Specific Antibodies in Domestic Cats during First COVID-19 Wave, Europe. *Emerg Infect Dis*. **2021**, *27*, 3115-3118. doi: 10.3201/eid2712.211252.

108. Calvet, G.A.; Pereira, S.A. Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de Janeiro, Brazil. *PLoS One*. **2021**, *16*, e0250853. doi: 10.1371/journal.pone.0250853.

109. Goryoka, G.W.; Cossaboom, C.M. One Health Investigation of SARS-CoV-2 Infection and Seropositivity among Pets in Households with Confirmed Human COVID-19 Cases-Utah and Wisconsin, 2020. *Viruses.* **2021**, *13*, 1813. doi: 10.3390/v13091813.

110. Zhao, S.; Schuurman, N. Serologic Screening of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Cats and Dogs during First Coronavirus Disease Wave, the Netherlands. *Emerg Infect Dis.* **2021**, *27*, 1362-1370. doi:10.3201/eid2705.204055.

111. Villanueva-Saz, S.; Giner, J. Serological evidence of SARS-CoV-2 and co-infections in stray cats in Spain. *Transbound Emerg Dis*. **2022**, *69*, 1056-1064. doi: 10.1111/tbed.14062.

112. Dias, H.G.; Resck, M.E.B. Neutralizing antibodies for SARS-CoV-2 in stray animals from Rio de Janeiro, Brazil. *PLoS One.* **2021**, *16*, e0248578. doi: 10.1371/journal.pone.0248578.

113. Bosco-Lauth, A.M; Hartwig, A.E. Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proc Natl Acad Sci U S A.* **2020**, *117*, 26382-26388. doi: 10.1073/pnas.2013102117.

114. Mattos-Silva, P.; Felix, N.S.; Silva, P.L.; Robba, C.; Battaglini, D.; Pelosi, P.; Rocco, P.R.M.; Cruz, F.F. Pros and cons of corticosteroid therapy for COVID-19 patients. *Respir. Physiol. Neurobiol*. **2020**, *280*, 103492. https://doi.org/10.1016/j.resp.2020.103492.

APPENDIX

Appendix I- Informed Consent Statement.

U.PORTO ipvc esa

DECLARAÇÃO DE CONSENTIMENTO INFORMADO

AO PARTICIPANTE / REPRESENTANTE:

Por favor, leia com atenção todo o conteúdo deste documento. Não hesite em solicitar mais informações se não estiver completamente esclarecido.

Exmº(ª) Senhor(a)

No âmbito do projeto do Mestrado em Enfermagem Veterinária de Animais de Companhia, a ser desenvolvido pela estudante Andreia Oliveira, orientada pela Prof Doutora Helena Vala e pelas co-orientadoras Prof. Doutora Teresa Mateus e Prof. Doutora Maria Pereira, a realizar no Instituto Politécnico de Viana do Castelo e Instituto Politécnico de Viseu, em parceria com a Universidade do Porto, pretende-se efetuar um estudo em SARSCoV-2 em animais de companhia, sobretudo em felídeos, para o qual se solicitou a colaboração dos Hospitais Veterinários sediados no Norte e Centro do País.

Na perspetiva *One health*, urge conhecer o estatuto imunitário da população de animais de companhia nas duas regiões, relacionando-o com as deslocações ocorridas no período pré e pandémico, numa abordagem pioneira, integrando pessoas, animais e ambiente nesta investigação.

Deste modo, solicita-se a colaboração de V. Exa para responder às questões de um inquérito e para a doação de uma amostra de soro do seu animal de companhia.

Mais se informa que tais práticas são isentas de risco e que ao participar pode beneficiar do conhecimento sobre o estado atual de imunidade contra a infeção por SARS-CoV-2 (COVID 19) do seu animal de companhia, sem que, no entanto, lhe seja atribuído qualquer benefício financeiro ou encargo.

As amostras biológicas serão estudadas no laboratório das instituições acima mencionadas e todos os dados recolhidos serão tratados com confidencialidade, pelo que a sua privacidade será respeitada e a sua identidade não será revelada. Os resultados serão comunicados, por email, a cada um dos tutores que colaboraram no estudo, com conhecimento do respetivo CAMV.

É ainda de salientar que os voluntários deste estudo estarão a contribuir para o avanço do conhecimento no que respeita à grave pandemia de COVID-19 que assola o mundo.

Informa-se que a participação é voluntária, podendo V. Exa. desistir a qualquer momento, sem qualquer consequência prejudicial discriminatória na relação com o seu CAMV e na prestação de cuidados de saúde ao seu animal, e que a sua privacidade será respeitada, sendo que todos os dados recolhidos serão confidenciais e a sua identificação estará apenas acessível ao profissional da saúde experiente que faz a recolha da amostra, pois os investigadores consagram como obrigação e dever o sigilo profissional.

Importa ainda lembrar que os investigadores deste projeto não têm qualquer benefício financeiro mas têm suporte oficial para realizar este estudo científico.

- Declaro ter compreendido os objetivos, riscos e benefícios do estudo, explicados pelo investigador que assina este documento;

- Declaro ter-me sido dada oportunidade de fazer todas as perguntas sobre o assunto e para todas elas ter obtido resposta esclarecedora;

- Declaro ter-me sido assegurado que toda a informação obtida neste estudo será estritamente confidencial e que a minha identidade nunca será revelada em qualquer relatório ou publicação, ou a qualquer pessoa não relacionada diretamente com este estudo, a menos que eu o venha a autorizar por escrito;

- Declaro ter-me sido garantido que não haverá prejuízo dos meus direitos se não consentir ou desistir de participar a qualquer momento;

Assim, depois de devidamente informado (a) autorizo a participação neste estudo:

Data:

Nome:

____

Assinatura do participante

Declaro que prestei a informação adequada e me certifiquei que a mesma foi entendida:

Nome do investigador: Andreia Oliveira

Data: 26 Janeiro 2021

ASSINATURA

andreia.oliveira@ipvc.pt

Escola Superior Agrária de Ponte de Lima, Instituto Politécnico de Viana do Castelo

Rua D. Mendo Afonso, 147

Refóios do Lima

4990-706 Ponte de Lima

Orientador: Helena Vala

Data: 26 Janeiro 2021

____

ASSINATURA

hvala@esav.ipv.pt

Escola Superior Agrária de Viseu, Instituto Politécnico de Viseu

Estrada de Nelas, Quinta da Alagoa, Ranhados

3500-606 Viseu

Portugal

Appendix II- Online questionnaire.

Topics	Question	Options	Type of Question
Owner's information	Owner's gender	Male/Female/I don't want to answer	Multiple choice
	Owner's age	From 18 to 30 years old/From 30 to 50 years old/From 50 to 70 years old/Over 70 years old	Multiple choice
	Owner's actual residence	Viana do Castelo/Braga/Vila Real/Bragança/Porto/Aveiro/Viseu Guarda/Coimbra/Castelo Branco Leiria/Lisboa/Santarém/Portalegre Setúbal/Évora/Beja/Faro	Multiple choice
	City of residence		Open-ended
	Owner's Academic qualifications	Elementary School (1st Cycle) Elementary School (2nd Cycle) Elementary School (3rd Cycle) Secondary Education/Graduation Bachelor's degree/Master's Degree PhD (Doctorate)/Other	Multiple choice
	Owner's professional activity	Student/Employed/Unemployed Retired	Multiple choice
	Number of members in the household		Open-ended
	Do you have other pets?	Yes/No	Dichotomic
	If the answer is yes, please list the species	Dog/Cat/Ferret/Birds/Other	Multiple choice
	How does the household normally interact with your cat?	Not much interaction (feeding, watering, cleaning the cat's litter box)/ Play, petting, and cuddling/Rested on the lap/Share the bed/Share the sofa/Other	Multiple choice
	How often do you usually give attention to the cat?	All day long/During a part of the day, e.g., in the evening/Only in some moments of the day/We do not usually provide any attention to the cat/Other	Multiple choice
Cat	Status	Non-neutered/Non-spayed/Neutered/Spayed	Multiple choice
	Gender	Female/Male	Dichotomic
	Age	Under 1 year/From 1 to 5 years/From 5 to 10 years/More than 10 years	Multiple choice
	Breed	Persian/Maine Coon/Siamese/Scottish Fold/Norwegian Forest/British Shorthair/Bengal/ European Shorthaired /Other	Multiple choice
	Is the cat mainly indoor (stays inside) or outdoor (have access to the road)?	Indoor/Outdoor/Indoor and Outdoor	Multiple choice

	Do you usually vaccinate your cat once a year?	Yes/No	Dichotomic
	What about deworming? How often do you do it?	Every 3 months/Every 6 months/Every year/When possible/I do not usually do it	Multiple choice
	Did your pet show any unusual clinical signs and/or behavior change since the beginning of the pandemic?	Yes/No	Multiple choice
	If the answer is yes, please specify which ones	Respiratory disease (coughing, runny nose, sneezing, runny eyes, dyspnea)/Apathy/ Fever/Digestive disorders (diarrhea, vomiting)/Skin problems/Loss of weight/ Loss of appetite/Behavioral disorders/ Conjunctivitis/Other	Multiple choice
	Does your animal present any chronic disease?	Yes/No/I don't know	Multiple choice
	If the answer is yes, which one/what one?	Diabetes/Kidney/hepatic disease/Joint disease/ FIV/FeLV/Heart disease/Hypothyroidism /Gingivostomatitis/Other	Multiple choice
	If the answer is yes, is monitoring therapy performed for that chronic disease?	Yes/No	Dichotomic
	If the answer is yes, what is the name of the medicine?		Open-ended
COVID-19	Has the owner been diagnosed for COVID-19?	Yes/No	Dichotomic
	Has another member of your household been diagnosed with COVID-19?	Yes/No	Dichotomic
	How was the diagnosis performed for COVID-19?	With nasopharyngeal or oropharyngeal swab for PCR test/With nasopharyngeal or oropharyngeal swab for rapid test/With blood sampling for serological test (antibodies)	Multiple choice
	During which period(s) did you obtain positive test(s)? You can select more than one option:	1st wave (April 2020-September 2020)/2nd wave (October 2020-December 2020)/3rd wave (from January 2021)	Multiple choice
	During the time when the household member was living with COVID-19, did he/she interact with the cat?	As usual/Reduced interactions/Reduced interactions and used protection (mask, gloves) while interacting/Did not interact/Other	Multiple choice

Appendix III- Characterization of household, including demographics and household composition (n=94, otherwise n value is presented).

	Characteristics		Percentage (%)
Demographics	Sex	Female	86.2
		Male	13.8
	Age (years)	18-30	21.3
		30-50	43.6
		50-70	29.8
		>70	5.3
	Educational qualification	Basic education	20.2
		High school	26.6
		Degree	28.7
		Master	17.0
		Other	7.5
	Professional activity	Employed	73.4
		Retired	14.9
		Unemployed	7.4
		Student	4.3
	District of residence	Porto	50
		Évora	13.8
		Braga	12.8
		Viseu	5.3
		Aveiro	5.3
		Beja	4.3
		Setúbal	3.2
		Portalegre	3.2
		Viana do Castelo	1.1
		Faro	1.1
Household	Human membres	1	14.9
		2	38.3
		3	21.3
		4	21.3
		Other	4.2
	Pets (n=79)	Dog	58.2
		Cat	38.0
		Bird	3.8

Appendix IV- Characterization of sampled cats. n=176, otherwise n value is presented.

Characteristics			Percentage (%)
Signalment	Sex	Female	52.8
		Male	47.2
	Reproductive status	Fertil	6.8
		Non-fertil	93.2
	Age (years)	1-5	45.5
		5-10	39.2
		>10	15.3
	Breed	European shorthair	84.7
		Siamese	5,7
		Persian	4.5
		Norwegian Forest cat	0.6
		Other	4.5
Environment	Lifestyle	Indoor	77.8
		Indoor/oudoor	20.5
		Outdoor	1.7
	Other animals	Yes	91.5
		No	8.5
	Co-habitant pets (n=161)	Dog, cat	48.4
		Cat	30.4
		Dog	9.3
		Other	11.9
Clinical status	Annual vaccination	Yes	78.4
		No	21.6
	Deworming	Yes, every 3 months	68.8
		Yes, every 6 months	15.9
		Yes, annualy	8.5
		Ocasionally	4.5
		No	2.3
	Chronic disease	No	80.1
		Yes	12.5
		Don`t now	7.4
	Type of chronic disese (n=22)	Kidney/liver disease	22.7
		Retroviral infection	22.7
		Heart disease	18.2
		Gingivostomatitis	9.1
		Hipotiroidism	4.5
		Other	22.7
	Medication (n=9)	Food suplements (oral hygiene, feline idiopathic cystitis, behavior, immunomodulators)	66.7

		Non-steroidal anti-inflammatory drugs	22.2
		Steroidal antiinflamatory drugs	11.1
	Clinical signs or behavior changes during the pandemic, and type (n=31)	No	82.4
		Yes	17.6
		Behavioral changes	67.7
		Weight loss	9.7
		Respiratory clinical signs	9.7
		Appetite loss	6.5
		Digestive clinical signs	6.6
		Conjuntivitis	6.5
		Fever	3.2
		Skin problems	3.2
		Other	6.5
Interaction with humans	Before pandemic, type of interaction	Playing, petting, and cuddling	96.0
		Rest on the lap	72.2
		Share the bed	65.9
		Share the sofa	64.8
		Other	13.7
	Before pandemic, frequency of interaction	All day	48.3
		Part of the day	46.0
		Ocasionally	6.25
		Did not interact	0.57
	After household human COVI-19 diagnoses (n=36)	As usual	86.1
		Reduced interaction	11.1
		Did not interact	2.8