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SARS-Corona Virus Type-2 Detection of Cohabiting Feline with COVID-Positive Individuals in Bandung, Indonesia

Roostita Lobo Balia [a,b], Fauzi Rohman [c], Okta Wismandanu [a,b], Lidya Chaidir [d,e], Tyagita [b,d,*], Pranyata T. Waskita [b,c], Vesara A. Gatera [f], Sarasti Windria [b,d], Mas Rizky A. A. Syamsunarno [d,e], Gemilang L. Utama [g,h]

[a] *Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Sumedang, 45360, Indonesia*
[b] *Veterinary Medicine Study Program, Faculty of Medicine, Universitas Padjadjaran, Sumedang, 45360, Indonesia*
[c] *Master Program in Biotechnology, Graduate School, Universitas Padjadjaran, Sumedang, 45360, Indonesia*
[d] *Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Sumedang, 45360, Indonesia*
[e] *Research Center for Translational Biomaker Research, Universitas Padjajaran, Sumedang, 45360, Indonesia*
[f] *Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Padjajaran, Sumedang, 45360, Indonesia*
[g] *Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Bandung, Sumedang, 45360, Indonesia*
[h] *Center for Environment and Sustainability Science, Universitas Padjadjaran, Sumedang, 45360, Indonesia*
Corresponding author: [*]tyagita@unpad.ac.id

***Abstract*— Since people and domesticated animals have lived together for a long time, it is possible that diseases could be spread by accident, as happened with SARS-CoV-2. There have been reports of cats in Italy, Spain, and France being exposed to SARS-CoV-2. Not much is known about how farmed animals were exposed to SARS-CoV-2 in Indonesia, which was named the epicenter of COVID-19 in July 2021. The study's goal was to determine if SARS-CoV-2 was present in felines living with people who had COVID-19 in the Bandung, Indonesia, area. Nineteen felines were used in the study. These felines came from seven people who had tested positive for COVID-19. For RT-qPCR testing, samples were taken from the nose, oropharynx, and rectal areas. Blood sera were taken for quick IgM/IgG antibody tests for SARS CoV-2. Using RT-qPCR on nasopharyngeal samples from the felines being studied, it has been seen that four of them have tested positive. But it is interesting to note that only one of these people could be found using a rectal test. There was no clear sign of antibody formation when IgM/IgG rapid test results from blood samples were looked at. The felines that showed positive results were very close to their caretakers and had symptoms that were similar to those of influenza. The results of our study show that there is a chance that SARS-CoV-2 could be passed on to felines who live with people who have COVID-19. Because of this finding, more study needs to be done in this area.**

***Keywords*—Feline; nasopharyngeal swab; rectal swab; SARS-CoV-2; Indonesia.**

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I. INTRODUCTION

COVID-19 (the novel coronavirus disease) was initially detected towards the conclusion of 2019 in Wuhan, People's Republic of China. Subsequent to its emergence, the ailment has rapidly disseminated to an astounding 220 nations across the globe. The documented instances have been identified in excess of 63 million laboratories, with a consequential fatality count of over 1.4 million individuals [1]. The etiology of the ongoing COVID-19 pandemic can be attributed to the pathogenicity of a Beta virus. The viral agent, characterized by its family Coronaviridae, is positive-sense with single-stranded RNA genomes [2,3]. The aforementioned RNA virus harbors a genome spanning from 26 to 32 kilobases, rendering it the most extensive of its category. It has been previously documented by sources [4,5].

Currently, seven distinct coronavirus strains have the potential to impact Homo sapiens, with nCoV-2019 being among this cohort. After thoroughly examining four distinct types, it has been determined that they elicit only mild symptoms. The nomenclature of these species is HCoV-HKU1, HCoV-NL63, HCoV-229E, and HCoV-OC43, as per the relevant literature [6,7]. It is imperative to bear in mind that Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome

Coronavirus (MERS-CoV) have been identified as the etiological agents of fatal pandemics. As of 26 April in the year 2023, the urban center of Bandung in Indonesia has documented a cumulative sum of 105,293 cases that have been confirmed, with a daily influx of 471,110 new cases and a total of 1,487 fatalities, as reported in reference [10].

It is noteworthy to observe a rising inclination among the populace of Indonesia to consider felines as indispensable constituents of their domestic milieu. The role of felids in the cultural milieu of Indonesia has undergone a metamorphosis from being a purely ornamental feature to assuming a pivotal function in the quotidian activities of the populace. The alteration in perspective can be attributed to the burgeoning prevalence of social media and the enlarging cohort of animal enthusiasts. Currently, in Indonesia, the prevalence of feline companionship has exceeded that of canine companionship, with a rate of 37% and 16%, respectively. Indonesia's exceptional perspective on owning domesticated animals sets it apart from other nations in Asia, where dogs remain the most prevalent choice for household companions [11].

As the global pandemic persists, it has been brought to our attention that COVID-19 is not solely impacting human populations. In March 2020, the initial instance of zoonotic contagious of SARS CoV-2 from animal to human was identified. This occurrence transpired after identifying the virus in a felid of considerable size at the Bronx Zoo, situated in the urban metropolis of New York City, located within the United States of America. The feline appeared to have come into contact with an individual who had contracted the pathogen above.

Furthermore, empirical data indicates that a pair of domesticated felines residing within a household in the United States had exhibited slight indications of respiratory afflictions [12]. There have been reported instances of SARS-CoV-2 infections in the feline population since the beginning of the current global pandemic. As of the fifteenth day of March in 2021, a total of one hundred and twenty-four instances of feline ailment have been documented globally. Numerous instances of these cases have been documented across a multitude of nations and continents.

A minimum of four rationales warrant contemplation regarding the contagion risk for COVID-19 between humans and animals or conversely. Insufficient evidence precludes definitive attribution of SARS-CoV-2 zoonotic origins to bats. It is noteworthy that alternative intermediate hosts may also be considered [14,15]. Due to the prolonged cohabitation of humans and domesticated animals, infectious disease transmission has been prevented [16]. It has been observed that a functional receptor for SARS-CoV-2, namely Angiotensin Converting Enzyme 2 (ACE2), is present in the gastrointestinal tracts of felids and tigers [17]. It has been reported that SARS-CoV-2 exposure has been observed in felines and canines in Italy, Spain, and France. Regrettably, the frequency of cross contagion of SARS CoV-2 from domesticated to feral fauna in Indonesia, a nation that has been designated as the COVID-19 pandemic ganglion as of July 2021, remains largely elusive. [19,20]. The primary objective of the investigation was to detect the presence of SARS-CoV-2 antigen in the oropharyngeal and rectal regions, as well as SARS-CoV-2 antibody in the blood serum of felines residing in Bandung, Indonesia, whose owners had been found positive for COVID-19.

II. Material and Method

Felines were sampled from households that tested positive for COVID-19 (Figure 1), who subsequently referred their companion animals to a private veterinary clinic, or from domiciles that provided expedient access to their animal companions within the confines of their abode. Nineteen feline subjects were subjected to nasopharyngeal and rectal swabbing after exposure to COVID-19-infected humans within the past fortnight. The feline health status was duly noted, encompassing any indications of SARS-CoV-2 infection, vaccination records, and prior interaction with their respective owners.

In the present context, close contact encompasses felid individuals who inhabit outdoor environments or are confined within a restricted enclosure. During the sampling procedure, the felines were subjected to the administration of xylazine at a dosage of 0.015 ml per body weight, aiming to induce anesthesia. Subsequently, intravenous extraction of their blood was performed. After a short interval of storage at room temperature, the blood specimen was subjected to the centrifugal force of 1800 g for a duration of 10 minutes to obtain the serum fraction. The methodology employed for identifying, detecting, and anticipating security measures is germane to the COVID-19 testing protocols [21].

Fig. 1 The figure shows nasal swab to detect the SARS-CoV-2 virus for diagnosing COVID-19 of the suspected cat. Diagnostic test results from a suspected cat sample

The swab specimens were aseptically placed into 3 milliliters of viral transport medium, namely the CITOSWAB VTM collection and transportation kit. In July in the year 2021, the esteemed Central Laboratory of Universitas Padjajaran conducted the preparation of samples and extraction of RNA utilizing a biosafety level-3 (BSL-3) laboratory. The viral RNA was obtained using a column-based RNA/DNA extraction kit (catalog number SPM-48, Da-An Gene Co., Ltd.). Subsequently, we employed a real-time reverse transcription polymerase chain reaction (RT-PCR) approach that specifically targeted the open reading frame 1ab (ORF1ab) and nucleocapsid (N) genes, utilizing the PCR Fluorescence Probing technique (Catalog number DNC-96, Da-An Gene Co., Ltd), under the manufacturer's prescribed protocols, to assess the integrity of the SARS CoV-2 specimen. The UJI COVID-19 IgG/IgM kit (Catalogue Number AUC-10D20, manufactured by Pakar Biomedika Indonesia) was employed to assess the efficacy of the SARS-CoV-2 antibody.

III. Results and Discussions

A. Results

Upon careful examination of the data presented in Table 1, it has come to our attention that among the cohort of 19 felines under investigation, a total of four individuals (namely, Cat7, Cat10, Cat12, and Cat13) have yielded positive results for SARS-CoV-2 upon conducting nasal, oropharynx, and rectum swabs, as illustrated in Figure 1, Figure 2, and Table 1.

TABLE I
THE NASAL, OROPHARYNX AND RECTUM SWAB OF SUSPECTED FELINES CHARACTERISTICS OF FELINES WITH POSITIVE COVID-19 OWNERS IN BANDUNG, INDONESIA

Felines	Swab		
	Nasal	Oropharynx	Rectum
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	+	+	+
8	-	-	-
9	-	-	-
10	+	+	+
11	-	-	-
12	+	+	+
13	+	+	+
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-
19	-	-	-

Notably, the incidence rate observed in the present study surpasses that of the study conducted in Spain, wherein SARS-CoV-2 was detected in 12.5% of the felids scrutinized. Two felids that underwent SARS-CoV-2 testing (Cat7 being asymptomatic, while Cat10, 12, and 13 exhibiting flu-like symptoms) had intimate associations with their respective owners, involving physical proximity and cohabitation. The felids can potentially contract SARS-CoV-2 from asymptomatic carriers [23]. Notably, out of the 38 RT-qPCR swab data, twelve were deemed inconclusive, resulting in a percentage of 31%. It is plausible that the inconclusive outcomes could be attributed to the sole detection of either the N-Gene or Orf1lab. The N-gene, situated at the apex of the genetic hierarchy, confers protection to the enclosed genetic material by ensconcing it within a protective shell. Acknowledging that the N-Gene plays a crucial role in safeguarding the viral RNA gene center in the context of SARS-CoV-2 is imperative. It is imperative to highlight that the predominant gene of this virus is Orf1lab, which exhibits open reading frames with overlapping characteristics [24,25]. The indeterminate outcomes may have arisen due to SARS-CoV-2 identification fragments, contaminants, or a procedural aberration during the initial phase of sample handling. Given that we have conducted RT-qPCR on two separate occasions and obtained concordant outcomes, it appears plausible that there were no technical aberrations during the RT-qPCR procedure.

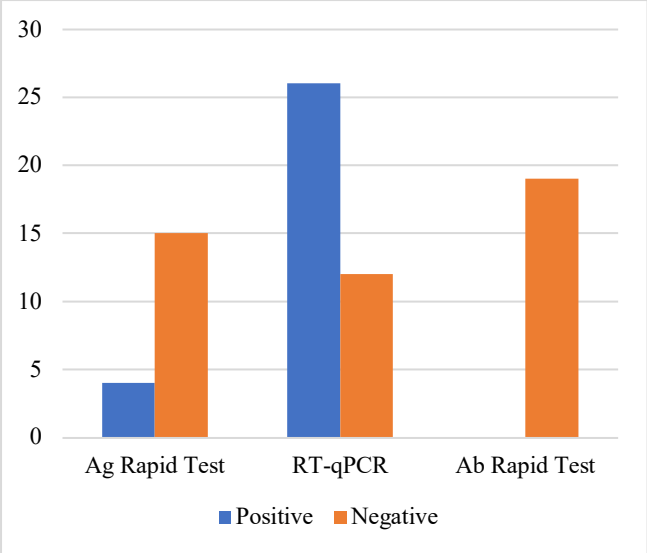

Fig. 2 Diagnostic test results from a suspected cat sample

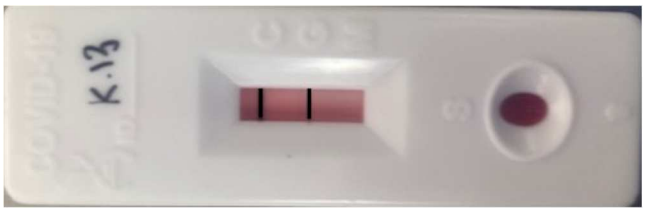

Fig. 3 Positive results marked by 2 lines appearance of the kit, indicate the presence of viral antigens.

Regrettably, the serological assay failed to yield any affirmative outcomes. The findings suggest that the rapid antibody detection kit could not identify the occurrence of SARS-CoV-2 antibody, and that felids may have exhibited no antibody-antigen response to SARS-CoV-2 or that the antibody had already undergone clearance from their bloodstream. The findings of a prior investigation have demonstrated that felids possess neutralizing antibodies against SARS-CoV-2, with titers ranging from 1/20 to 1/1080. In order to elucidate the intricacies of the antibody-

antigen interplay of SARS-CoV-2, particularly in the context of domesticated fauna, further experimentation is imperative.

B. Discussion

The risks for zoonotic animals to humans' transmission of COVID-19, particularly in the context of companion animals such as canines, felines, and other domesticated species, underscores the need for heightened attention to this matter. This issue calls for further examination and reflection within the domain of epidemiology. It has been duly noted that the SARS-CoV Type 2 virus exhibits a significant affinity towards domesticated *Canidae* and *felidae* species that cohabit with at least one individual diagnosed with the virus, as per the latest research in the field of environmental health. As mentioned earlier, the statement postulates the risks that the disease could be passed from animals to humans or vice versa [27]. Scholars have postulated that the diverse suppositions regarding the genesis of SARS-CoV-2 are presently unverifiable or falsifiable. Upon conducting a thorough phylogenetic analysis, it has been ascertained that SARS-CoV Type 2 exhibits a significantly heightened degree of genetic resemblance, amounting to 96%, with two SARS-like coronaviruses, namely bat SL CoVZX45 and bat SL CoVZX21.

Notably, both of these coronaviruses are naturally harbored by chiropteran species, which underscores the importance of understanding the complex interactions between wildlife and human health. The SARS-CoV Type 2 pathogen exhibits a proclivity for binding to particular receptors, thereby facilitating its invasion of horseshoe bats and civets. It has been duly noted that the susceptibility of gallinaceous avifauna, porcine taxa, anatine avifauna, and murine rodents to this particular pathogen appears to be inconsequential [23]. It is widely acknowledged within the scientific community that the Feline Coronavirus (FCoV) group acts as the precursor virus of the Feline Infectious Peritonitis Virus (FIPV), a pathogen that is highly prevalent among domesticated felines worldwide [31]. On the contrary, a proposal has been posited positing that the origin of group II Feline Coronavirus (FCoV) may be ascribed to the amalgamation of canine and feline CoVs. From an epidemiological perspective, it appears that this hypothesis is relatively less plausible and demonstrates a significant biological and molecular pathogenesis compared to cases involving Feline Coronavirus type I. As a senior professor in environmental health, it is important to consider all possible factors and analyze the evidence thoroughly before drawing any conclusions.

The results of a recent investigation suggest that the contagion of the SARS-CoV Type 2 virus in felines is not of significant concern. Regarding felines, it is noteworthy that the transmission of the disease exhibits a remarkable degree of resilience, thereby facilitating contagion to susceptible felines through droplet dissemination. It has been duly noted that a considerable proportion of animal species that are susceptible to SARS-CoV Type 2 exhibit modifications in various amino acids of their ACE2 proteins, as per the current scientific literature. It is hypothesized that these specific taxonomic groupings, particularly those in close proximity to human habitation, demonstrate an increased vulnerability to contracting the SARS-CoV Type 2 pathogen. It is conceivable to postulate that in the event of a robust establishment by one of these organisms, it may potentially act as a reservoir for the dissemination of disease among other wildlife, as has been previously proposed in scholarly literature [34]. After conducting sequencing research on ACE2 from several mammals, it has been observed that the domestic cat (*Felis catus*) displays the highest degree of sequence similarity, with an overall identity of 85.2%. It is noteworthy to observe that the *Manis javanica*, commonly known as the Malayan pangolin, and Oryctolagus cuniculus, which is referred to as the European rabbit, display a remarkably analogous genomic constitution, with a similarity rate of 84.8% between the two taxa. The comparative analysis of these findings has been conducted under established protocols and methodologies in the field of zoonoses research, utilizing the human ACE2 sequence.

The latest scientific findings have demonstrated that the SARS-CoV Type 2 pathogen ACE2 as a receptor for cellular entrance, as has been evidenced. It has been duly noted that the felid species demonstrate a comparatively elevated expression of ACE2, particularly in their tissues, digestive tract, and urinary system, in contrast to the human populace. Notably, felids demonstrate a greater inclination towards acquiring and transmitting illnesses through various means, in contrast to canines [37].

It has been postulated with significant frequency that domestic felids represent the domesticated species that are most commonly affected by SARS-CoV Type 2 infection. The findings of the RT-PCR analysis reveal the detection of SARS-CoV-2 in six felines that have been procured from various geographical regions, including France, Spain, Chile, Hong Kong, and New York. Within the cohort of six felines presently under investigation, a noteworthy proportion of four individuals have displayed clinical manifestations, including emesis, diarrhea, dyspnea, asthenia, anorexia, cough, sneezing, and ocular discharge. Following the confirmation of the individual's positive status for Covid 19, clinical manifestations were observed on the seventeenth day of the disease course.

There have been several documented instances of COVID-19 infections in both human and non-human animal populations, including felids. It has been postulated that felids may have acquired the infection from their human caregivers who were asymptomatic at the time, as per the literature citation 23. It is anticipated that future research endeavors will augment our understanding of the transmission dynamics of the disease, particularly in the realm of zoonotic transmission. It is of utmost importance to provide additional clarification in order to develop and execute effective preventative measures aimed at reducing the transmission of COVID-19 [35].

Regarding transmission, it is essential to recognize that the SARS-CoV-2 virus employs a receptor similar to that of the respiratory mucosa. This phenomenon increases the likelihood of zoonotic virus transmission [36]. A recent investigation suggests that SARS-CoV-2-infected felids are capable of passing the virus to other felids through direct contact. Nevertheless, the extent of their potential to pass the virus to other animals or humans remains unclear, necessitating additional research [40]. Due to the homology of nucleotides and amino acids within the spike protein, it is

plausible that inter-animal transmission could occur despite the uncertainty surrounding the concept of zoonotic virus transmission. Notable similarity exists between the genomes of SARS-CoV Type 2 and other animal coronaviruses. The nucleotides may constitute the genetic material changed during transmission to animal hosts. It is plausible that variations in the amino acid sequences may have resulted, possibly imparting increased virulence to certain taxa, particularly those containing the spike protein.

According to the evidence, the likelihood of zoonotic transmission appears relatively low. Given the remarkable similarity between the nucleotides and amino acids of the spike protein of SARS-CoV Type 2 and those of other animal coronaviruses, it is plausible that the virus could be transmitted between species. It is possible that nucleotide mutations occurred during zoonotic transmission of the virus to animal hosts expressing spike protein-associated amino acids, thereby increasing the pathogenicity of the virus in said animal hosts [10].

In the context of SARS-CoV and SARS-CoV-2 infection in cats, it is notable that the clinical manifestations are typically limited to moderate respiratory symptoms. It has been observed that felids infected with SARS CoV-2 exhibit viral RNA in their pulmonary organs and gastrointestinal tracts for up to 6 days and 3 days, respectively, following infection. In addition, it is noteworthy that on the third post-infection day, viral ribonucleic acid was detected only in the pulmonary tissue of these felids. The relevant information is documented in reference 35. However, there have been documented instances of viral transmission via the fecal-oral route [41]. The pathogen SARS CoV-2 primarily causes infections that are restricted to the upper respiratory tract. The general public has hypothesized that feline infections can be attributed to their human caregivers. However, no empirical evidence has established the plausibility of interspecies or zoonotic transmission of infections. [35]

According to previous research, the present findings indicate that felids that have been exposed to sick individuals in the past may have detectable levels of SARS-CoV-2 [22]. According to the findings of reference 41, SARS CoV-2 has the propensity to replicate efficiently in feline species and can be transmitted between felines via droplets. Given the ambiguity surrounding the transmission dynamics of the virus between humans and felines, or vice versa, it is imperative to emphasize that the positive results cannot be interpreted as conclusive evidence of feline susceptibility to SARS-CoV-2. If the answer is affirmative, the potential transmission of SARS-CoV-2 from human caregivers to their feline counterparts should be considered as an alternative scenario. It is highly recommended to conduct additional in vitro analyses using gastrointestinal tract-derived cell lines to address this issue.

During the course of our investigation, we encountered a number of obstacles. The limitations of our investigation included several factors, such as the sample size, which, despite being adequate, could have been larger. In addition, the number of individuals who owned SARS-CoV-2-infected cats was limited, limiting the generalizability of our findings. The timing of specimen collection was also problematic, as it affected both PCR and serologic results. In addition, the lack of a feline-specific COVID-19 IgG/IgM test was a significant limitation we faced. The imposition of a curfew in the study area restricted the mobility of our personnel, which constituted an additional difficulty. This has substantially hindered our collaborative efforts to investigate potential human-animal transmission routes.

Our findings indicate that physical contact or cohabitation between an infected cat owner and another feline companion may increase the likelihood of pathogen transmission. Multiple studies have shown that feral felids are susceptible to contracting diseases from contaminated surfaces or asymptomatic pathogen carriers. However, the etiology of these instances of contagiousness remains obscure [43].

IV. Conclusion

It has been noted that felines can display clinical indications suggestive of SARS-CoV-2 infection when their owners, who have been verified to be suffering from COVID-19, undergo laboratory analysis. Unfortunately, the scarcity of examples in this study diminishes its usefulness to some extent. Notwithstanding the elevated incidence of COVID-19 cases in the city of Bandung, Indonesia, the acquisition of samples for diagnostic testing has presented a formidable obstacle. Moreover, it has been observed that certain individuals affected by the COVID-19 disease, whose diagnosis was confirmed by a laboratory, have chosen not to reveal their condition to the relevant authorities owing to the unfavorable stigma linked with the ailment. As a senior professor in environmental health, it is imperative to emphasize the significance of transparency and timely reporting of infectious diseases to prevent further transmission and safeguard public health. It is of utmost importance for individuals who are custodians of *Felis catus* to exercise prudence, given the proclivity of these domesticated quadrupeds to roam freely and cohabit close to Homo sapiens. It has been empirically established that the implementation of efficacious hygiene practices, the enforcement of social distancing measures, the maintenance of physical separation, and the adherence to quarantine protocols are all efficacious in mitigating the risk of transmission. The incorporation of whirlpools, footbaths, disinfectants, and hygienic walls necessitates careful deliberation.

Based on the existing corpus of knowledge, it seems highly unlikely that zoonotic transmission of SARS-CoV-2 will transpire. It is important to consider that the possibility of transmission occurring bidirectionally cannot be disregarded. This observation is in line with the findings previously reported by scholars [16]. Through the analysis of serological, molecular, and trial inoculation studies, it has been determined that the SARS-CoV-2 virus possesses zoonotic potential in select animal species. The conclusive demonstration of the transmission of SARS-CoV-2 between animals and humans or vice versa remains uncertain. In light of the present global scenario, it is imperative to adopt a comprehensive strategy to ensure the protection of domesticated animals and captive wildlife from the persistent COVID-19 outbreak. In light of the ongoing COVID-19 pandemic, it is imperative that we extend the same level of care and attention to our animal companions as we do to our human family members in order to mitigate the spread of this highly contagious virus. It is of utmost importance to limit

interpersonal contact with individuals outside the boundaries of one's dwelling and to confine domesticated felines indoors in order to prevent the potential for other companion animals to stray. Adorning our animal companions with masks is ill-advised as it may potentially jeopardize their welfare. It is of utmost importance that individuals who are suffering from a communicable disease abstain from any form of physical interaction with domesticated animals. It is of utmost importance to abstain from participating in behaviors such as osculation, embrace, or sharing a sleeping space with non-human animals, among other similar activities. In the event of an individual contracting SARS-CoV-2, it is recommended that they seek other household members' aid in caring for their pets. If the former alternative is not practicable, it is recommended that individuals wear masks while interacting with their animal companions. It is of utmost importance that the owner takes necessary measures to ensure that their animal companion is provided with nutritionally appropriate sustenance. It is important that companion animals displaying signs of illness or potential COVID-19 infection be expeditiously transported to a veterinary facility for prompt evaluation and management.

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