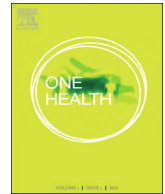




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Editorial Commentary

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

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1. Introduction

Infection with the new coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), termed coronavirus infectious disease 19 (COVID-19), came to the attention of health workers in December 2019 when a cluster of people who attended the seafood and animal market in Wuhan, Hubei Province, China presented with a severe respiratory illness [1,2]. The outbreak, first localized to Wuhan, quickly spread to neighbouring provinces and within three months was declared a pandemic, with cases reported in nearly 200 countries in all regions of the world. As of 8 April 2020, the pandemic has resulted in over one million four hundred thousand confirmed cases and over 76, 000 deaths, including over 655,000 cases and 51, 000 deaths in Europe. While China has not registered new cases for several days, parts of western Europe have become the foci of the COVID-19 pandemic. The close association between humans and their pets has led to an examination of the potential risks of transmission. Viral RNA has been detected in two dogs and two cats, belonging to SARS-CoV-2 infected owners, in Hong Kong and Liège, Belgium. While pale in comparison with the scale of infection in the human population, these observations have nonetheless provoked many violent reactions towards dogs and cats.

More recently, National Veterinary Services Laboratories (NVSL) of the US the Animal and Plant Health Inspection service (APHIS) in Ames, Iowa confirmed that a female Malayan Tiger at the Bronx zoo had positive RT-PCR for SARS-CoV2. Bronx Zoo veterinarians confirmed that the Malayan tiger, her sister, two Amur tigers, and three African Lions had developed a dray cough [3].

The objective of this note is to inventory the risks of SARS-CoV-2 contamination of pets, especially dogs and cats, by their owners, and the potential role of pets in the ongoing COVID-19 pandemic.

2. Discovery of two dogs and two cats testing positive for SARS-CoV-2

To date, two dogs and two domestic cats have tested positive for SARS-CoV-2.

The first dog was a 17-year-old Pomeranian Loulou, belonging to a 60-year-old woman who began to develop COVID-19 symptoms on February 12, 2020, and was admitted to the hospital on February 24. She returned home on March 8, after recovering from the infection. Saliva and runny nose samples were collected from the dog on three occasions, February 26, 28, and March 2, 2020, and analyzed by specific quantitative PCR. Small amounts of viral RNA were detected in all three samples, indicating that the samples collected had a low viral load [4]. Subsequently, a serological screening, carried out on a blood sample collected on March, 3, 2020, revealed the presence of specific anti-SARS-CoV-2 antibodies [5]. Thus, it appears that the dog was infected with the virus and had developed a specific immune response. The dog died on March 16, 2020, without ever having developed symptoms consistent with COVID-19. The death of this very old animal was attributed to kidney and heart failures. The low viral titres observed in this dog suggest it had developed a low-productive infection, and the likelihood of infectious transmission was minute or nonexistent.

The second dog is a German shepherd belonging to a German resident in Hong Kong who became ill with COVID-19 symptoms at the beginning of March. When the owner tested positive for COVID-19 infection, the dog was quarantined and tested, along with another dog from the same residence. This dog also tested positive for quantitative PCR and, as of March 25, had still not developed any clinical signs [6]. The viral sequences obtained from the dog and its owner were found to be identical, confirming the hypothesis of direct contamination of the animal from its owner. In addition, the other mixed-breed dog did not express disease and PCR RNA detection was negative. As with the first

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dog, the infection of the German Shepherd was low positive and probably non-contagious.

The third case is a pet cat belonging to a SARS-CoV-2 infected person who developed digestive (anorexia, diarrhea, vomiting) and respiratory (cough, difficult breathing) symptoms one week after the return of its owner to Belgium from Italy [7]. Nine days after the onset of these symptoms, the person's health improved. Tests of the cat with conventional, qualitative PCR detected SARS-CoV-2 RNA in successive samples of feces and gastric fluids, which was further confirmed by next-generation sequencing. While these results clearly demonstrate the presence of viral RNA, quantitative PCR analyses, and serological tests are needed to understand this infection and potential risks better. Like the dogs, the cat was asymptomatic, and there was no evidence it had developed a productive and infectious infection.

The last case involves a domestic short-haired cat in Hong Kong [8] living with a SARS-CoV-2 infected owner. Immediately following the 30 March 2020 confirmation of the owner's infection, the cat was quarantined and oral cavity, nasal, and rectal samples collected for testing using quantitative PCR. Again, SARS-CoV-2 RNA was detected from these samples, and like the first cat, this animal did not develop any signs of disease.

3. The case of wild felids housed in the Bronx zoo, New York City

Five Siberian tigers (*Panthera tigris*) and three African lions (*Panthera leo*) are housed in two enclosures at the Bronx Zoo in New York City. On March 27, 2020, a four-year-old tiger, named Nadia, developed a dry cough and some wheezing. As the symptoms persisted, samples were collected from the tiger on April 2, 2020, and she tested positive for SARS-CoV-2 [3]. By April 3, three additional tigers and all of the lions exhibited cough and a loss of appetite. None of the animals were in respiratory distress. The lions and tigers were isolated, and no other animals at the zoo have shown any signs of respiratory disease. As of April 6, 2020, the presumably infected animals are stable and recovering, though they have not all been tested.

4. Has the massive outbreak led to transmission to pets?

Europe is home to a tremendous number of dogs and cats. Indeed, a study by Fédération des Fabricants d'Aliments pour Chiens, Chats, Oiseaux et autres animaux familiers (Facco)/ Taylor Nelson Sofres (TNS Sofres), conducted in 2016 among 14,000 household's representative of French households, reported a population of about 7.3 million dogs and 13.5 million cats, and that nearly 50% of households have at least one dog or cat. With similar numbers likely for other European countries, and given the close association between pets and their owners, the question inevitably arises of whether these pets have been contaminated as the virus has spread. Similarly, the American Veterinary Medical Association estimates that in the USA, the percent of pet-owning households at 57–59%, with dogs constituting 38.4% and cats 25.4% [9].

5. Favorable modes of virus transmission

The modes of transmission of SARS-CoV-2 are favorable for human contamination to dogs and cats. Although the routes of contamination are not completely identified, the virus is transmitted mainly through respiratory droplets emitted during coughing, sneezing, talking, or breathing. Recent observations also indicate transmission through fomites by hand-to-mouth, eye conjunctiva or by touching the nose with hands contaminated by saliva or respiratory droplets [10]. As a result, in addition to common habitation, licking and kissing are additional risk factors facilitating transmission of the virus between sick people and their pets.

6. Coronaviruses infecting humans and carnivores are phylogenetically closely related

SARS-CoV-2 belongs to the *Coronaviridae* family, in the order *Nidovirales*. The members of *Nidovirales* (from the Latin *nidus*, “nest”) earn their name because they all use a distinctive “nested set” transcription strategy in which the expression of the genes that code for viral proteins are “nested” as subgenomic sequences [11]. The Coronavirus genome consisting of positive-sense, single-stranded, polyadenylated, nonsegmented RNA.

The *Coronaviridae* comprise two subfamilies, including *Coronavirinae*, whose members are commonly referred to as coronaviruses (CoVs). This subfamily is subdivided into four genera, *Alphacoronavirus* (alpha-CoV), *Betacoronavirus* (beta-CoV), *Gammacoronavirus* (gamma-CoV) and *Deltacoronavirus* (delta-CoV) [12,13]. To date, only seven coronaviruses have been reported in humans, four of which are ubiquitous with seasonal circulation and mostly causing relatively mild colds (HKU1, NL63, OC43 and 229E). The other three, of more recent zoonotic origin, are associated with severe acute respiratory syndromes, namely SARS-CoV, MERS-CoV and now SARS-CoV-2 [14]. Of these seven human coronaviruses, NL63 and 229E belong to the alpha-CoV genus, while the other five are included within the beta-CoV genus. Coronaviruses detected in dogs and cats also belong to these two viral genera [15,16]. Like SARS-CoV-2 and the other respiratory syndrome viruses, the canine respiratory coronavirus (CRCoV), responsible for a respiratory pathology in dogs, belongs to the beta-CoV genus. The S protein of CRCoV, responsible for attachment to the host cell membrane, has nearly 97% sequence similarity with that of HCoV-OC43, probably due recombination [17]. Canine coronavirus (CCoV) and Feline coronavirus (FCoV), both responsible for digestive diseases, belong to *Alphacoronavirus*.

7. SARS-CoV-2 may jump the species barrier easily

SARS-CoV-2 and SARS-CoV are two viruses belonging to two closely related groups, they are biologically comparable, and both have a zoonotic origin indicative of their ability to cross the species barrier. In addition, the discovery of several SARS-CoV-2-related viral sequences in several species of *Rhinolophus* bats as well as Malayan pangolins (*Manis javanica*) has led to the hypothesis that the zoonotic origin of the SARS-CoV-2 is likely from one of these species [2,18], but with significant difference in the receptor-binding domain of the spike protein such that the Spike protein of RaTG13 coronavirus of the bat *Rhinolophus affinis* does not bind well to the human receptor [19]. While the Spike protein of SARS-CoV2 does bind well to the ACE2 of humans, ferrets, cats, and other species that have high receptor homology [20]. In addition to the wide variety of SARS-CoV-related coronaviruses reported in *Rhinolophus* bats (including bats sampled in Europe), closely related viruses have also been found in masked-palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*), two species in the order of *Carnivora* which includes cats and dogs [21–24]. The diversity of species susceptible to SARS-CoV and SARS-Cov-2 related viruses strongly suggests a propensity of these viruses to cross the species barrier, particularly in the context of the frequent encounters between carnivores and others small mammals that may facilitate interspecies transmission.

8. Spike protein plasticity and interspecies transmission

The coronavirus genome encodes 16 major non-structural proteins (nsp1 to nsp16), and four major structural proteins. The structural proteins include the nucleocapsid (N) protein which associates with genomic RNA, and the envelope-constituting spike (S) protein, membrane (M) protein and envelop (E) protein (some coronaviruses also have a hemagglutinin esterase (HE) structural protein). Protein S is a membrane glycoprotein, consisting of two subunits, which is involved

in the attachment of the virus to the host cell, membrane fusion, and entry into the cell [25]. The globular S1 subunit allows the virion to attach to the target cell via an interaction between an approximate 100 amino acids receptor binding domain (RBD), and a receptor located on the surface of the host cell (for example angiotensin-converting enzyme 2 (ACE2) in the case of SARS-CoV-2). The S2 subunit promotes virion fusion with the host cell membrane. The S1 subunit, and the RBD, in particular, is the most variable part of the coronavirus genome. Because of its high genetic variability and its primary role in host cell attachment and infection, the RBD is strongly associated with the cellular tropism and host spectrum of the virus. A very recent study has shown that the SARS-CoV-2 RBD, including sites directly attaching to the ACE2 receptor of human cells, is almost identical to SARS-CoV RBD [19,20,26]. Only six amino acids appear responsible for SARS-CoV and SARS-CoV-2 attachment to the ACE2 receptor and may, therefore, be important in determining the host spectrum of these two viruses. Based on structural and biochemical properties, the SARS-CoV-2 RBD would not only have an affinity for the human ACE2 receptor but also for that of several animal species, including pets (dogs, cats, and ferrets) and farm animals (cattle, sheep and horses) [27]. The apparent plasticity of the S protein, and in particular the RBD, may allow coronaviruses to initially bind and then adapt to ACE2 protein receptors of different species, giving them a higher likelihood of SARS-CoV2 crossing the species barrier [19]. Thus, many animals could be infected with SARS-CoV-2 and serve as intermediate hosts in the spread of the virus. In the context of the ongoing COVID-19 pandemic, the possibility of dogs and cats becoming infected and participating in the spread of the virus should be addressed.

9. Genetic characteristics with high evolutionary potential

The two major modes of evolution causing changes in the coronavirus genome are the drift or natural selection of mutations and the exchange of genetic sequences from other viruses through recombination. The RNA genome of coronaviruses is one of the largest known, ranging in size from 27,000 to 31,000 nucleotides. The genome's large size promotes the appearance of point mutations, deletions, or insertions, which can sometimes lead to the emergence of new variants with different phenotypic characteristics. These mutations are significant, even though SARS-CoV2 has some proofreading capability. Specifically, the RNA-dependent RNA polymerase (RdRp) of SARS-CoV2, which is remarkably similar to that of SARS-CoV and MERS-CoV [28] has a limited proofreading capability. Coronaviruses, including SARS-CoV2 encode a proofreading 3'-to-5' exoribonuclease distinct from the viral RdRp, which imparts some fidelity during reproduction, enhance virulence, and by resisting some mutations, frustrates efforts at using some antiviral drugs that rely on mis-incorporations during RNA synthesis [29].

In addition, numerous studies have shown, in several animal species, the existence of frequent genetic recombination events in coronaviruses. Genetic recombination is an exchange of genetic material that can be autologous, when the exchange occurs between viruses affecting the same host species, or heterologous when the exchange is between viruses affecting different host species. For example, recombination of the S protein appears to be a common feature of some coronaviruses that are pathogenic to cats and dogs [30,31]. Similarly, genetic recombination events within human CoVs are well documented [14]. The high prevalence of dog infections with canine coronaviruses in Europe [32] might foster recombination with SARS-CoV-2 if an animal were infected with both viruses. Such an event, if it were to happen, could lead to the emergence of a new coronavirus with unpredictable phenotypic characteristics (transmissibility and virulence). However, the likelihood of such a scenario is difficult to assess.

10. Conclusion

Epidemiological, biological and virological characteristics of coronaviruses, particularly their demonstrated ability to easily cross species barriers, suggest that pet contamination by sick owners is not only likely but expected given the numerous opportunities for spill-over during a massive outbreak. This concern is highlighted by a recent experimental study which, while performed using non-natural conditions, in particular, that newborn animals were infected with a very high viral load, showed that SARS-CoV-2 has the ability to infect several different pet species [33]. While viral shedding from pets does not appear sufficient to in turn infect other family members or other animals encountered during walks, the usual precautionary measures should be urgently considered as part of a global control and 'one health' approach. Finally, it is imperative that further studies be quickly carried out in order to better establish the risk of contamination of pets from humans as well as the risk that infected pets have as source of infection for humans.

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