Canine SARS-CoV-2 infection

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Case Report

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Abstract

SARS-CoV-2 emerged in Wuhan in December 2019 and has caused the pandemic respiratory disease, COVID-19. Following what is presumed to be an initial zoonotic transmission event, the virus is now spreading efficient in humans. Very little is known about the susceptibility of domestic mammals kept as pets to this virus. Samples were collected over a 13-day period from a 17 year-old neutered male Pomeranian in Hong Kong SA that was taken into isolation after two members of the household tested positive for the virus. Nasal swabs were consistently positive on the five occasions the dog was tested using quantitative RT- PCR with viral loads between 7.5xE2 to 2.6 x10E4 RNA copies per mL of sample. The dog remained asymptomatic. Cultures attempted on three RT-PCR positive nasal samples were negative. Gene sequences from samples from two household members were identical. The viral sequence from the dog differed at three nucleotide positions; two of these resulted in amino acid changes but their significance is yet to be determined. Seroconversion was not detected but this was expected given the asymptomatic infection and low virus load. The evidence suggests that this is an instance of human-to-animal transmission of SARS-COV-2. It is likely that we could see similar events in other infected households. We do not have information yet on whether this virus can cause illness in dogs but no specific signs were seen in this dog. Whether infected dogs could transmit the virus to other animals or back to humans remains unknown. In this case it did not appear to have occurred.

Introduction

SARS-CoV–2 emerged in Wuhan in December 2019 and has caused a global epidemic of a respiratory disease now named as COVID–19.[1] Following what is presumed to be an initial zoonotic transmission event, the virus is now spreading via efficient human-to-human transmission, with cases identified in over 100 countries and territories.[2] Very little is known about the susceptibility of domestic mammals kept as pets to this virus. In 2003, the related SARS coronavirus was shown to have infected domestic cats and a dog in the outbreak in Hong Kong SAR in Amoy Gardens with prolonged shedding of virus in cats.[3] However, no evidence was presented that domestic animals played any role in onward transmission of the SARS outbreak.

When any new case of COVID–19 is diagnosed in Hong Kong SAR, the household contacts, regarded as “close contacts” are quarantined for COVID–19 in designated centres. Pet owners are given the option of having their dogs and cats looked after and isolated by the Hong Kong Agriculture, Fisheries and Conservation Department (AFCD). If permission is granted by owners, samples are taken so as to build knowledge on whether pet animals in contact with infected humans have been infected with this virus and to assist in determining the best methods for managing animals in quarantine. As of 11th March three dogs and one cat have been quarantined and tested. One of the dogs tested positive for SARS-CoV–2 RNA on multiple occasions over a 13 day period.

Case Results

A 60 year-old woman who developed symptoms on the 12th February 2020 was diagnosed with COVID–19 disease on 24 February 2020. She kept a 17 year-old neutered male Pomeranian which had a number of pre-existing diseases, including a Grade II heart murmur, systemic and pulmonary hypertension, chronic renal disease, hypothyroidism and a previous history of hyperadrenocorticism (Dr Florence Chan pers comm). One female domestic helper (secondary case A) in the household developed a fever on 16th February and was subsequently confirmed to be infected. The remaining three members of the household, who were not known to be infected were sent to a quarantine centre on 26th February. That evening, the dog was transferred to a holding facility managed by AFCD and nasal, oral, and rectal swabs as well as a faecal sample were collected. Additional specimens for virus detection were collected from the dog on 28th February, 2nd March, 5rd March and 9th March. A blood sample was collected on 3rd March for serological testing.

Throughout the period in quarantine the dog remained bright and alert with no obvious change in clinical condition. On 7th March a saliva sample was collected from the second domestic helper (secondary case B) who was asymptomatic. Specimens from the three patients were confirmed to be SARS-CoV–2 positive by RT-PCR at the Public Health Laboratory Centre and viruses genetically sequenced (Institutional Review Board approval UW20–168). Until admission to the quarantine centre or hospital on 26 February, the two domestic helpers shared responsibility for daily care of the dog. (Figure 1).

Nasal and oral swabs collected from the dog on the 26th February were positive by quantitative RT-PCR for SARS-CoV–2 RNA while rectal and faecal specimens were negative. Initial results obtained at AFCD’s Tai Lung laboratory were confirmed by additional tests on the same samples done at the School of Public Health, The University of Hong Kong (HKU), a World Health Organization reference

laboratory for SARS-CoV–2 diagnostics. The positive samples were RT-PCR positive for six gene-sequence targets of SARS-CoV–2 (see Table 1). AFCD used assays for the E and rdrp gene sequences (TIB Molbiol Lightmix® Modular Assays). HKU assays for nsp14 and N gene that detect SARS-CoV, SARS-CoV–2 and bat SARS- CoV (2) and assays for nsp16 and M gene that are specific for nCoV with no reaction with SARS-CoV.[4] On subsequent sampling occasions, the nasal swabs remained positive throughout, the oral swabs tested positive on the first two samplings (on 2nd March only E gene was positive). Rectal swabs and faecal specimens remained negative throughout. Nasal swabs had higher viral load (lower CT values) than the oral swabs.

Attempts to culture the RT-PCR positive nasal swab samples collected on three occasions (28th February, 2nd & 5th March) on Vero-E6 cells gave negative results. Given the low viral load (range 7.5xE2 to 2.6 x10E4 RNA copies per mL of specimen) it was unlikely that virus culture would be successful. In human patients with COVID19, virus isolation was not successful when viral load in the specimen was <10E6 per mL.[5] The serum sample collected from the dog on 3 March 2020, together with 20 control samples from unaffected dogs were tested in micro-neutralization test for SARS-CoV–2. All samples, including that of the infected dog tested negative for neutralizing antibody. In the absence of invasive disease in the dog, it is not surprising that the dog remained sero-negative. It is known that patients with mild or asymptomatic MERS-CoV infection fail to develop antibody responses.[6]

Viral RNA from the nasal swab specimen collected from the dog on 26th February was genetically sequenced and compared with the virus found in clinical specimens from the owner and the secondary case A. Full virus genome sequence (29,764 nucleotides) was obtained from the index case and secondary case A and 27,871 nucleotides (94% of genome; from nucleotide position 1933 to 19,803) was obtained from the initial nasal swab of the dog (Table 2) (Supplementary Fig 1). The viral sequences from the index case and the first domestic helper were identical across the full genome. The viral sequence from the dog differed at 3 nucleotide positions, viz. A6,567G within nsp3 gene, A18,950G within nsp14 and C29,730T within 3'UTR region. The mutations at positions 6567 and 18,950 were non- synonymous leading to amino acid changes D6567G and K18950R respectively. Interestingly, there was nucleotide heterogeneity at position 6567 in the dog virus sequence with 41% of sequence reads being A as found in the index case while 59% were G (sequence coverage >500 fold). Partial gene sequencing was conducted on the sample from secondary case B covering the region of the nucleotide changes seen in the canine sample but the nucleotide residues were identical to those found in the index case rather than the dog.

Discussion And Conclusions

Our results suggest infection of this dog by SARS-CoV–2, most likely acquired from one of the infected humans in the household. The likelihood that the positive results represent environmental contamination is considered unlikely for the following reasons. The dog was removed from the potentially contaminated premises on the 26th February but remained RT-PCR positive for virus RNA for at least 12 days thereafter. Given mucociliary clearance of nasal secretions, it is not likely that contamination will persist at levels detectable by RT-PCR for such a long time. Furthermore, if environmental contamination was the explanation for these findings, it is more likely to be acquired by licking of contaminated surfaces and thus oral swabs would be expected to have higher viral load than the nasal swab, which was not the case. Thirdly, the viral sequence from the dog, though similar, was not identical to that of the humans who were potential sources for the infection of the dog.

ACE–2 is known to be the receptor for this virus. Canine ACE2 is similar to that of humans (Supplementary Figure 2). Of 18 amino acids that are known to be involved in interaction between ACE–2 and the spike receptor binding domain (RBD) of SARS-CoV–2, there are five that differ between humans and dogs, but none of these are in regions known to disrupt the interaction between the RBD of SARS-CoV and ACE–2.[7] Direct evidence of experimental infection of dogs or ex vivo tissues of dogs with SARS-CoV–2 is awaited.

Given that there are no mutations detected between the index case and the two household contacts who were infected, the three mutations observed in the dog are notable, especially because two of these three are non-synonymous. It is possible that they arose through selection pressure of the interspecies transmission. The D1283G mutation in nsp3 is located at the domain known as betacoronavirus specific marker (βSM), a domain unique in betacoronaviruses.[9] The K304R mutation is located the N7-Mtase domain, a residue that is not conserved amongst betacoronaviruses. It is within the α1' domain of N7-Mtase which is known to be essential for Cap binding in SARS-CoV.[10] The C to A mutation is at the 3'UTR of SARS-CoV–2. The 3'end of coronavirus is believed to form secondary RNA structure to control virus RNA synthesis.[11] Too little is known of the functional relevance of any of these mutations to ascribe any relevance to inter-species transmission if any and this requires further investigation.

Taken together the evidence suggests that this is an instance of human-to-animal transmission of SARS-COV–2. It is likely that we could see similar events in other infected households. We do not have information yet on whether this virus can cause illness in dogs but no specific signs were seen in this dog. Whether infected dogs could transmit the virus to other animals or back to humans remains unknown. While the quantities of virus found in oral and nasal swabs in this dog were low, it is also possible that this represents the tail end of an infection that could have occurred a week or more before samples were collected, if the dog was infected soon after clinical disease commenced in the owner and first domestic helper. As more pet animals are tested in and outside Hong Kong SAR we will gain a greater understanding of the behaviour of this virus in pets and also find out whether they play any role in transmission of the virus.

These findings and the results from animal testing during the SARS outbreak in 2003 have implications for the management of mammalian pets owned by persons who develop SARS- CoV–2 infection. From a precautionary point of view, it would be ideal if these pets are held in isolation and tested virologically, as was done in this instance. However, facilities for such an approach may not be widely available. If the contacts of the case are quarantined within the home, it is advisable that the pet is also isolated within the same household. However, if all household contacts are removed to separate quarantine facilities, as occurred in this instance, it is necessary to be cognizant of a potential risk of transmission from infected dogs and advice needs to be tailored on a case-by-case basis.

The findings also have implications for future zoonotic transmission events by the precursor virus of SARS-CoV–2. Rhinolophid bats are considered a likely reservoir of the precursor of SARS-CoV–2 (12). However, based on experiences with SARS virus, it is likely that intermediate hosts serve to bridge transmission from bats to humans. Dogs can be sold in or found in the vicinity of wild-game animal markets, the presumed source for the initial zoonotic spill-over of SARS-CoV–2. They should be tested during investigations into the origin of this virus to determine if they play any role in spillover events.

Declarations

Ethics

The sequencing is done as part of the overall public health response in contact tracing and investigating cluster. Thus does not require IRB as long as patient confidentiality is maintained. Note, this is virus sequence – not human genome sequence which may have privacy implications. However, we do have an IRB approval number, which is UW20-168.

Author contribution

TS, ET, MP, LS, CB were responsible for design of the study, Monitoring and collection of samples from the dog was undertaken by ES and VY, and data and samples from humans was curated by DNCT, Molecular diagnostics was undertaken by PL,SMI, KT and DKWC, Virus genetic sequencing was undertaken by DKWC, LLMP and MP, LS and TS drafted the manuscript. Data analysis was undertaken by all authors who critically reviewed that manuscript.

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Tables

Table 1: RT-PCR testing results on nasal and oral swabs of the dog*

			TLVL laboratory				HKU laboratory								
			E gene (Ct) nsp16 gene (Ct)		RdRP gene (Ct)		nsp14 gene (Ct) M gene (Ct)			N gene (Ct and viral load)					
TLVL Case no.	Date of collection	Date of testing	Nasal	Oral	Nasal	Oral	Nasal	Oral	Nasal	Nasal N gene copies/mL	Oral	Nasal	Oral	Nasal	Oral
20-02756	26-Feb-20	27-Feb-20	33.9	34.52	38.97	Negative	36.76	37.96	34.71	1.2xE4	36.48	37.94	39.25	36.91	37.95
20-02824	28-Feb-20	28-Feb-20	31.98	36.07	37.44	Negative			34.58	1.1xE4					
20-02927	2-Mar-20	2-Mar-20	31.69	Negative	40	Negative			33.2	2.6xE4					
20-03046	5-Mar-20	5-Mar-20	33.58	Negative	38.53	Negative			38.43	7.5xE2					
20-03251	9-Mar-20	9-Mar-20	30.07	Equivocal	40.00	Negative	35.86	Negative	34.97	7.7xE3	Negative	36.96	Negative	36.24	Negative

**Faecal and rectal swab samples at each of these sampling occasions tested negative and not included in the table.*

Table 2: Nucleotide and amino acid substitutions in transmission between humans and to dog

	Nucleotide position		
	6548	18934	29716
Case\Gene	nsp3	nsp14	3'UTR
Index case	A (99.4%)	A (99.2%)	C (99.9%)
Secondary case A	. (99.7%)	. (99.1%)	. (100%)
Dog	A (41%) G (59%)*	G (100%)	T (99.9%)
	Amino acid position		
Index case	D	K	
Secondary case A	.	.	
Dog	G/D	R	

Bracket indicates percentage of the nucleotide occurring in sequencing reads with coverage >500 times
*: Nucleotide "G" found in 58.8% in the sequencing reads and "A" in remaining 41.2%.

Figures

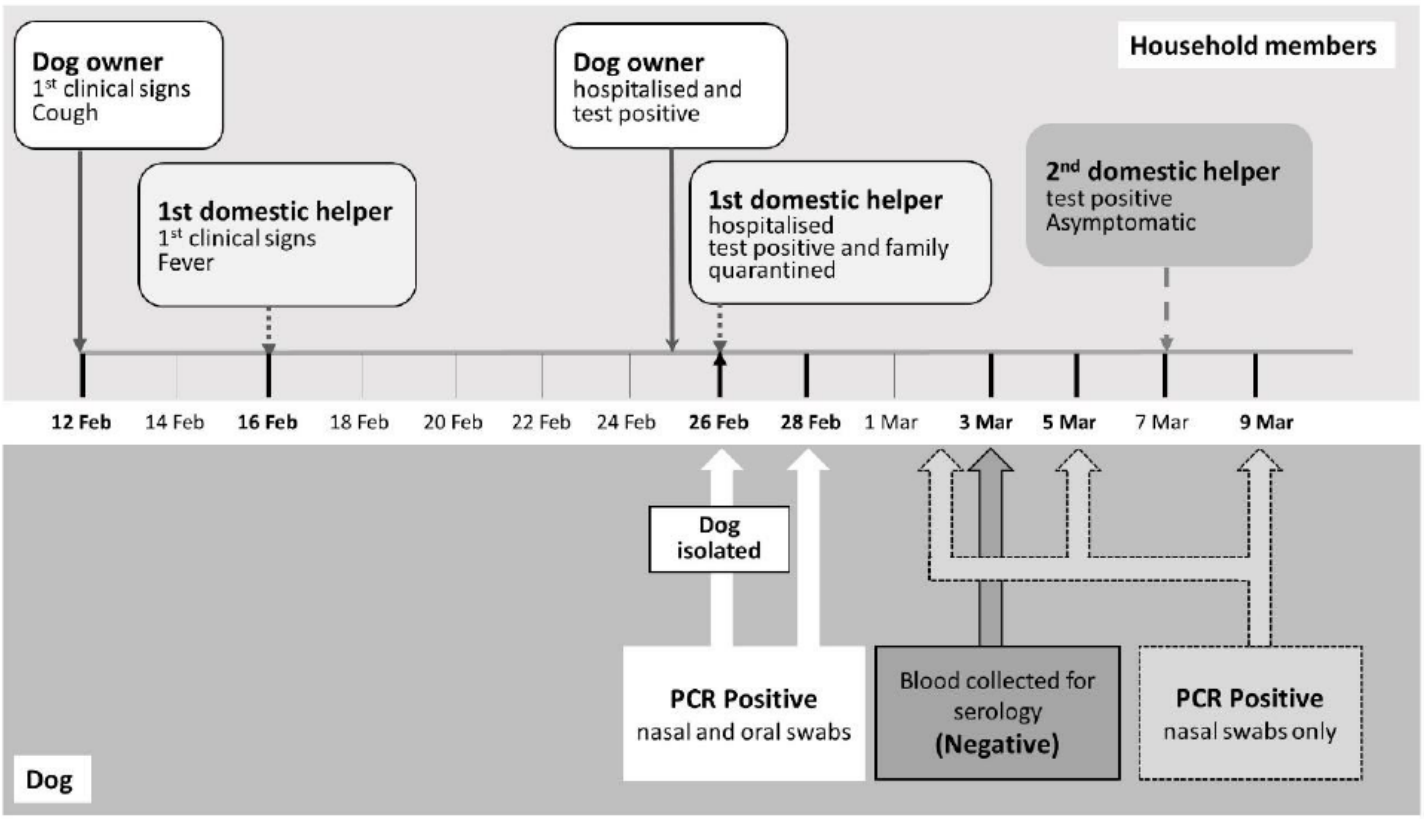

Figure 1

Time line of events

Supplementary Files

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- Supplementaryinformation.pdf