Transmission of SARS-CoV-2 (variant Delta) from pet hamsters to humans and onward human propagation of the adapted strain: a case study

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Appendix file

Abstract

Background:
Transmission of SARS-CoV-2 from humans to other mammals, including pet animals, has been reported. However, with the exception of farmed mink, there is no previous documentation that these infected animals can infect humans, nor of further onward spread among humans. Following a confirmed SARS-CoV-2 infection of a pet store worker, animals in the store and the warehouse supplying it were tested for evidence of SARS-CoV-2 infection.

Methods:
Viral swabs and blood samples from pet animals were collected in a pet shop and the warehouse supplying it and tested by SARS-CoV-2 RT-PCR and serological assays, respectively. SARS-CoV-2 RT-PCR positive samples were studied by full genome sequencing analysis.

Findings:
Over 50% of individually tested Syrian hamsters in the pet shop (8/16) and warehouse (7/12) were positive for SARS-CoV-2 infection in RT-PCR or serological tests. None of dwarf hamsters (n=77), rabbits (n=246), Guinea pigs (n=66), chinchilla (n=116) and mice (n=2) were confirmed positive in RT-PCR tests. SARS-CoV-2 viral genomes deduced from human and hamster cases in this incident all belong to Delta variant of concern (AY.127) that had not been circulating locally prior. These sequences are highly similar, but distinct. The viral genomes obtained from hamsters are phylogenetically related with some sequence heterogeneity and phylogenetic dating suggest infection in these hamsters occurred around 21 November 2021. Two separate transmission events to humans are documented, one leading to onward household spread.

Interpretation:
Pet hamsters can be naturally infected in “real-life” settings. The virus can circulate within hamsters and lead to human infections. Both genetic and epidemiological results strongly suggest that there were two independent hamster-to-human transmission and that such events can lead to onward human transmission. Importation of infected hamsters was the most likely source of virus infection.

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Research in context

Evidence before this study:
Transmission of SARS-CoV-2 from humans to different mammalian species, including pet animals, have been reported. However, the only example of such viruses being transmitted back to humans has been from farmed mink. Hamsters can be experimentally infected with SARS-CoV-2 and the virus can transmit between hamsters in experimental settings.

Added value of this study:
This study reveals that pet hamsters can acquire SARS-CoV-2 infection in real-life settings and can transmit the virus back to humans. The SARS-CoV-2 circulating in hamsters can allow sustainable virus transmission in humans. Our work highlights that some pet animals can be a secondary reservoir of SARS-CoV-2. It also suggests that the pet animal trade may be a pathway that can facilitate the movement of SARS-CoV-2 across national borders.

Implications of all the available evidence:
This study expands our understanding of the secondary animal reservoirs of SARS-CoV-2 in real-life settings. Awareness and appropriate quarantine and control policies are needed to reduce these reverse zoonotic and zoonotic events.

Introduction

SARS-CoV-2 and its descendent variants have demonstrated a wide host range besides humans. Natural human-to-animal infections have been documented in companion animals (dogs, cats, ferrets)[1-3], captive animals in zoos (feline species[4] and gorillas), farmed animals (mink)[5] and wild animals (white-tailed deer)[6]. Experimental challenge has identified that non-human primates, hamsters, ferrets, American minks, cats, dogs, racoon dogs, North American deer mice, Egyptian fruit bats, Asian small clawed otters, and white-tailed deer were highly susceptible to SARS-CoV-2 infection[7] (OIE: https://www.oie.int/app/uploads/2021/11/en-factsheet-sars-cov-2-20211025.pdf). Animal-to-animal transmission has been observed in hamsters[8], ferrets[9], cats[10], minks[5], racoon dogs[11], fruit bats[12], deer mice[13], and white-tailed deer[6]. Sustained transmission and continuous evolution of SARS-CoV-2 in animal species have been documented in the large mink farm outbreaks[5] and in the white-tailed deer populations in USA[6]. So far, zoonotic transmission has only been shown for the mink-adapted SARS-CoV-2 variant during the mink farm outbreaks in Denmark where large numbers of infected animals were housed in high density[14]. There has been no sustained human-to-human transmission of the mink-adapted variants.

SARS-CoV-2 virus may transmit between humans via multiple routes mediated by expelled respiratory fluids or exhaled aerosols that directly or indirectly reach the mucosal surface of a susceptible host. Experimental animal models have demonstrated transmission potential by direct contact (hamsters, ferrets, cats, racoon dogs, deer mice), by fomites (hamsters) or by aerosol (hamsters, ferrets, cats). Transmission in Syrian hamsters was more efficiently mediated via aerosols than via fomites[8]. Despite their high susceptibility to SARS-CoV-2, hamsters have not hitherto been documented to be infected outside of experimental settings.

Hong Kong has pursued a “zero-covid” strategy and has kept transmission at very low levels[15], with no locally acquired infections detected between 9-October-2021 to 8-January-2022, when variant Omicron was introduced via returning air-crew has led to multiple chains of local transmission. In particular, there were no locally acquired infections with variant Delta since 9-October-2021. None of the previous locally acquired Delta infections belonged to the viral lineage reported here (Pango lineage AY.127). All known AY.127 cases detected in Hong Kong prior to this involved incoming travelers, detected at the airport or in quarantine, with the last AY.127 case being detected in a quarantined traveler on 13-December-2022.

Here, we report an outbreak of SARS-CoV-2 variant Delta firstly identified in a pet shop worker on 15-January-2022. Subsequent investigation identified the source as pet hamsters imported from the Netherlands resulting in two independent zoonotic infections in humans and at least one further human-to-human transmission event in Hong Kong.

Methods

Sample collection

Samples were collected by veterinarians and technical staff led by the Government Agriculture, Fisheries and Conservation Department (AFCD). Full description of the method can be found in the appendix (p 2). RT-PCR positive samples collected from the initial screening investigations were tested and independently confirmed by two laboratories (AFCD and School of Public Health, HKU)

RT-PCR test

In brief, RNA from swab supernatant (140 µl) was extracted by using QIAamp viral RNA minikit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Eluted RNA (60 µl total) was then tested by quantitative RT-PCR assays specific for the ORF1b and N genes of SARS-CoV-2. Sequences for the primer-probe sets and RT-PCR conditions were described elsewhere[16]. Samples that were positive in both assays were classified as confirmed positives, whereas samples that were positive only in only one of these assays was classified as an inconclusive result.

Next generation sequencing

Representative SARS-CoV-2 positive RNA samples with adequate viral load were studied by next generation viral full genome sequencing techniques adapted from our previously described protocols[3,17]. Full description of the method can be found in the appendix (p 2). The deduced sequences are available at GISAID (Accession numbers: XXXXXX). The viral lineage was defined by the Pango nomenclature[18].

Phylogenetic analysis

Viral genomes deduced from this study were analyzed together with a set of representative sequences available in GISAID., including (1) the top 40 most similar AY.127 sequences (number of nucleotide divergence ranged from 4 to 9, compared to the local human index case sequence); (2) the top 5 most similar AY.127 sequences from Netherlands (number of nucleotide divergence ranged from 12 to 13, compared to the local index human case sequence); (3) all previous Hong Kong AY.127 sequences; and (4) outgroup reference sequences (from Pango lineages B, B.1.1.7, B.1.351, P.1, B.1.617.1); The public sequences of AY.127 lineage were retrieved from GISAID database on 2022-01-19. The outgroup reference sequences were retrieved from a pre-subsampled pre-aligned open database from Nextstrain (https://docs.nextstrain.org/projects/ncov/en/latest/reference/remote_inputs.html).

The Maximum likelihood phylogenies were estimated using IQ-TREE (v.2.1.3)[19], employing the TIM2+F+I nucleotide substitution model (best-fit model searched by IQ-TREE) with Wuhan-Hu-1 (GenBank: MN908947.3) as the outgroup. Dating of the tree was performed by using IQ-TREE LSD2 (PMID: 26424727). The node dates and confidence intervals were estimated by 100 times replicates, with specifications "–date-root 2019-12-26 –date-ci 100 –date-options \"-l −1\"." Ultrafast bootstrap[20] and SH-aLRT test [21] were performed to evaluate the support of tree branches.

Mutation analysis

The single nucleotide polymorphism (SNPs) among the studied consensus sequences were compared to the reference sequence (Genbank accession: MN908947.3) using ucsc-faToVcf (http://genome.ucsc.edu/cgi-bin/hgTrackUi?db=wuhCor1&g=nextstrainSamples) and annotated

by SnpEff[22]. The occurrences of single nucleotide variants were counted via https://cov-spectrum.org/.

Surrogate virus neutralization test (svNT)

SARS-CoV-2 specific svNTs were conducted as previously described and it has been validated for use in animals including hamsters[23]. The description of this method can be found in the appendix (p 2).

Role of the funding source

The funding sources have no role in this study.

Results

Table 1 summarises key events concerning the outbreak.

A 23-year-old female pet shop worker, previously vaccinated with 2 doses of Comirnaty (Date of 2nd dose: 16-September-2021), presented with sore throat and cough on 11-January-2022. She tested positive by RT-PCR on 15-January-2022 (C_t value: 21) and was confirmed to be a COVID-19 case on 16-January-2022 by a second confirmatory RT-PCR test. Full genome sequencing analysis revealed that the infection was caused by VOC Delta (AY127 virus lineage) (Figure 1). She had no known contact with other individuals known to be infected. She worked in a pet shop (Pet shop A) which sold hamsters, rabbits and chinchillas.

A mother (Patient 2) and daughter (Patient 4) visited pet shop A on 8 January 2022 where they met the index case and discussed matters relating to pet hamsters the daughter had previously purchased on 4-January-2022. The mother developed upper respiratory symptoms on the 12-January-2022, was tested positive by RT-PCR on 17-January-2022 and confirmed by a second RT-PCR test on 18-January-2022. Subsequently her husband (Patient 3), daughter (Patient 4) and son (Patient 5) were also confirmed to be SARS-CoV-2 RT-PCR positive (Table 1). All these infected individuals were previously vaccinated with 2 doses (Mother: 2nd dose of Coronavac in September-2021; Father: 2nd dose of Coronavac in August-2021, Son: 2nd dose of BBIBP-CorV in June-2021 and Daughter: 2nd dose of Comirnaty in July-2021).

During the initial screening investigation of the animals at the pet shop A carried out on 17-January-2022, 125 swab specimens collected from hamsters (n=69), rabbits (n=42) and Guinea pigs (n=14). Seven (10.2%) of the swabs from hamsters (species unspecific), but none of those from other animals were confirmed positive by RT-PCR (Table 2). The wholesale warehouse supplying this pet-shop chain was investigated on 18-January-2022, with 511 swabs collected from hamsters (n=137), rabbits (n=204), Guinea pigs (n=52), chinchilla (n=116) and mice (n=2) housed there (Table 2). One Syrian hamster swab was RT-PCR positive for SARS-CoV-2.

Since the initial screening sampling suggested that hamsters were infected at both the warehouse and the pet shop, a more detailed sampling of pet shop A and warehouse was carried-out on 18-January-2022 and 19-January-2022, respectively, with swabs and serum being collected from the Syrian and dwarf hamsters available there (Table 3). At the pet shop, 7 (43.8%) of 16 Syrian hamsters were confirmed to be RT-PCR positive with both screening and confirmatory tests while a further two were indeterminate RT-PCR positive with only the screening RT-PCR assay being positive but the confirmatory assay being negative. Five (31%) of 16 Syrian hamster sera were positive for SARS-CoV-2 antibodies. Overall, 8 (50%) of 16 Syrian hamsters had evidence of infection, either by serology or confirmed RT-PCR, with 4 animals tested positive by both serology and RT-PCR, 3 animals tested positive by RT-PCR alone (C_t values for N gene: 23.30, 30.38, and 37.43), and 1 animal tested positive by serology alone. A total of 3 cages housing Syrian hamsters were sampled and two (66.7%) had animals with confirmed RT-PCR or serology results. In contrast, none of 20 cages housing dwarf hamsters were positive in either RT-PCR or antibody assays. Since neutralizing antibody are readily detectable from hamsters as early as 5 days post-inoculation[24], the detection of 2 animals with viral RNA but without antibody suggests that infection may be a recent event.

Twelve Syrian hamsters and 55 dwarf hamsters, from 7 and 20 cages respectively, were sampled at the warehouse on 19-January-2022 (Table 3). Two (16.7%) of the swabs were RT-PCR positive (C_t values for N gene: 29.14 and 38.74) and seven (58.3%) of the sera had evidence of antibody. Seven (58.3%) of 12 Syrian hamsters had evidence of confirmed RT-PCR or serologically confirmed SARS-CoV-2 infection, with 2 animals tested positive by both serology and RT-PCR

and 5 animals tested positive by serology alone. Viral RNA can be detected in the nasal washes of experimental challenged hamsters for up to 35 days post-inoculation (H Yen, unpublished data). Although viral kinetics in oral swabs has not been determined, the detection of 5 animals with antibodies but without viral RNA suggest that infection may have occurred earlier. Among the 7 cages housing Syrian hamsters, 5 (71.4%) had infected animals. None of 55 dwarf hamsters from 20 cages sampled were positive in the confirmatory RT-PCR or serological test.

There was no evidence of overt illness in the hamsters sampled in pet shop A or the warehouse. As the warehouse supplied pet animals to other retail outlets in Hong Kong, five additional pet shops B to F were sampled on 19-January-2022 (Appendix, p 4). Two of 49 swabs from hamsters collected at one additional pet shop (C) was found to have confirmed evidence of SARS-CoV-2 RNA. Serum was not collected.

The hamsters at the affected warehouse were imported from Netherlands to Hong Kong in two different batches (arrival dates: 22-December-2021 and 7-January-2022). The consignment that arrived on 22-December-2021 was transported by Qatar Airways and transited in Doha, Qatar, involving change of aircraft, the transit time was around 15 hours. Water was topped up but no food was provided. This consignment had 96 rabbits, 990 *Phodopus sungorus* (white dwarf hamster) and 90 *Phodopus roborovskii* (roborovski dwarf hamster). The consignment that arrived on 7-January-2022 was transported by KLM which stopped over in Bangkok but without change of aircraft. The cargo hold was opened for off-loading the cargo designated for Bangkok but the animals did not leave the aircraft. No additional water or food was provided. The transport cages had a mesh covering, so contamination during transit cannot be excluded. This consignment had 116 rabbits, 720 *P. sungorus* (white dwarf hamster), 118 *Mesocricetus auratus* (Syrian hamster), 25 Guinea pigs and 30 chinchillas. The hamsters were initially kept in the warehouse on arrival and smaller consignments delivered to the retail shops. The warehouse did not operate on an all-in all-out basis. Some hamsters arriving on the 7-January-2022 were transferred to pet shop A on the day of arrival.

Specimens from the first 3 human cases (Patients 1-3) and positive hamster samples collected in pet shop A (n=11) and the warehouse (n=1) were subjected to full viral genome sequence analysis. The deduced viral genomes all belong to the Delta AY.127 viral lineage. These sequences are clustered together in the tree (Figure 1), indicating that these viruses are of the same origin.

The deduced sequences from these human and hamster cases are highly similar, but not identical. Viral genomes from patient samples differ from those from hamsters by 1 to 13 nucleotides (Appendix, p 5). The divergent date of this cluster of human and hamster viruses is estimated to be on 21-November-2021 (Appendix, p 3; 95% CI range: 18-October-2021 to 16-December-2021). Interestingly, the viral genomes of Patient 1 is phylogenetically distinct (5 nucleotides different) from those of Patients 2 and 3, which are identical (Figure 1). The virus from Patient 1 differs from that in Patients 2 and 3 by 5 nucleotides. However, some virus sequences from hamsters in pet shop A (Samples 1 and 10) only differ by 1 nucleotide with those of Patient 1. Patients 2 and 3 have viruses with genetic sequence closer (3 nucleotide difference) to hamster sample 7 in pet shop A. These results highly suggest that Patient 1 and Patient 2 independently acquired the infection from hamsters at the pet shop rather that having been infected by each other. As Patient 3 did not visit the pet shop, these findings further suggest that the SARS-CoV-2 virus circulating in hamsters allowed at least 1 human-to-human transmission.

The virus sequences in hamsters are genetically closely related to recent AY.127 viruses detected in multiple European countries. By contrast, none of the AY.127 sequences previously detected

from returning travelers in Hong Kong is genetically similar to the sequences detected in this outbreak. This further supports the hypothesis that this outbreak was caused by a recent introduction of AY.127 virus from Europe. Using some recent and genetically closely related European AY.127 viral sequences from humans as references, there are 4 unique non-silent mutations that can be reproducibly found in both studied human and hamsters cases (Table 4). Interestingly, 3 of these mutations are located in the spike viral protein, with 2 mutations in the N-terminal domain (NTD: L18F, H49Y) and one mutation in the receptor binding domain (RBD: D427G) in the S1 region. The L18F mutation can affect the binding of some NTD-specific antibodies[25] and the H49Y mutation can enhance viral entry[26]. The D427G is not located in the Receptor Binding Motif (RBM) that direct interacts with host ACE2[27], and its impact on ACE2 receptor binding and other biological functions require further investigation.

Discussion:
Our findings provide the first documented evidence of efficient animal-to-animal transmission of the Delta variant of SARS-CoV-2 in pet Syrian hamsters, hamster-to-human zoonotic jump and further onward spread between humans.

Specifically, we found that Syrian hamsters at a warehouse and two pet shops (A and C) supplied by this warehouse had evidence of SARS-CoV-2 infection. The viruses in hamsters in these three premises are genetically highly similar and they form a unique clade in the phylogenetic tree. However, these viruses were not genetically identical, suggesting that transmission in these hamsters had been ongoing for some time. The SARS-CoV-2 infecting Patient 1 who worked in pet shop A was highly similar to these hamster viruses, with only one nucleotide difference to some hamsters. Viral genetic analysis suggests that Patient 2 independently acquired infection from other hamsters in pet shop A and did not acquire infection from Patient 1. Thus, our findings suggest that there were at least two separate transmissions of virus from hamsters to humans. Given that viruses in hamsters is similar to the virus sequenced from the warehouse, and since both Patients 1 and 2 did not visit either the warehouse or pet shop C, the findings are highly suggestive that infection in Syrian hamsters in the warehouse was the source of infection in pet shops A and C and also of Patients 1 and 2. Taken together, the most likely conclusion is that both Patient 1 and Patient 2 acquired infection directly from infected hamsters in pet shop A. Patients 2 and 4 visited pet shop A on 4-January-2022 and again on 8-January-2022. Since Patient 2 developed symptoms on 12-January-2022, and given the mean incubation period of SARS-CoV-2 is around 5 days, it would be likely that she acquired infection from infected hamsters during her visit to the pet shop on 8-January-2022 rather than the hamster purchased on 4-January-2022. The alternative hypothesis that the index case got infected from an undetected human chain of Delta virus transmission within Hong Kong and then transmitted infection to hamsters in pet shop A, pet shop C and the warehouse is implausible, given the genetic diversity in the virus found in hamsters in the pet shop.

The source of infection of the warehouse remains to be definitively ascertained. The findings indicate that Syrian hamsters are the primary animal source in this outbreak as neither the dwarf hamsters nor other pet species sampled had evidence of infection. The viral genetic diversity observed in hamsters indicated that virus had been transmitting within this group of hamsters for some weeks, either at the warehouse or at a hamster farm that supplied the warehouse. Since Delta viruses had not been in circulation in Hong Kong for 3 months, importation of infected hamsters was therefore the most likely source of introduction of this chain of infection into Hong Kong. Although Omicron is increasingly becoming the dominant virus lineage in many parts of the world, Delta AY.127 lineage was predominantly found in Europe (including the Netherlands) (https://cov-lineages.org/lineage.html?lineage=AY.127). There were two shipments arriving at the warehouse in the previous month, but the shipment on 22-December-2021 only had dwarf hamsters while that on 7-January-2022 had only Syrian hamsters. Thus, the 7-January-2022 shipment was the most likely source of introduction. It was established that hamsters arriving on this shipment to the warehouse were supplied the same day to the pet shop A. The RT-PCR positive rates in pet shop A (7/16) and the warehouse (2/10) from sampling carried out on 18-January-2022 and 19-January-2022 were not significantly different (Fisher's exact test p=0.22), with evidence of active transmission among hamsters at pet shop A, i.e. detection of hamsters that are RT-PCR positive but seronegative. This further corroborated the animal-to-human transmission risk at pet shop A. However, the possibility of an undetected local chain of transmission in humans leading to infection of hamsters in the warehouse, though unlikely, cannot be excluded.

Spillover event from humans to mink and vice versa can occur in farm settings. This risk of mink to human transmission might be attributed to high-dose exposure of SARS-CoV-2 in farm with a high number and density of animals. There have been reports of zoonotic transmission of mink adapted SARS-CoV-2 to humans during large outbreaks of SARS-CoV-2 in mink farms in Europe[5,14]. Pet dogs and cats have been reported to acquire SARS-CoV-2 infection from infected human within the household but there is no evidence of transmission of virus back to humans[3,28]. This report is the first evidence of zoonotic transmission of SARS-CoV-2 from pets to humans and also of pet hamsters being infected naturally. Most importantly, the SARS-CoV-2 circulated in hamsters can achieve human-to-human transmission. This incident is also the first report of SARS-CoV-2 being transferred across international borders via the pet animal trade. There are other examples of viruses being moved across international borders via the pet trade, such as an outbreak of monkey pox in the USA attributed to importation of exotic animals from Africa[29].

In summary, we provide convincing evidence of pet hamsters naturally acquiring SARS-CoV-2 variant Delta and being the source of human infection. We also provide evidence suggesting the possibility of international movement of SARS-CoV-2 infection via the pet trade. The relatively low level of SARS-CoV-2 transmission in the period of this outbreak and its "One Health" approach to outbreak investigations likely allowed the detection and investigation of this outbreak. Similar events may be occurring, unsuspected, in many other parts of the world. These findings highlight that SARS-CoV-2 may be spilling over to other animal species unsuspected and providing a secondary reservoir for the virus for further adaptation and zoonotic spillover back to humans. The findings highlight the need for awareness, surveillance and for appropriate quarantine and control policies for the pet animal trade.

Contributors
THCS, HY, GML, MP, LLMP conceptualized the study and provided supervision; SSYC (AFCD), KWST, WS, SFS, KTC facilitated or conducted field investigations; CJB, PYTL, SMSC, HG curated the data; SSYC (HKU), LDJC, DYMN, PK, GYZL, MMYH, SYH, SPNL, AWYT, JCTK, LY did the laboratory work; HY, GML, MP and LLMP acquired funding; HY, GML, MP, LLMP wrote, reviewed, and edited the manuscript. All authors critically reviewed and approved the final version. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Data sharing:
Viral genomes deduced from this study are deposited GISIAD. Other dataset is available on request by contacting the corresponding author.

Declaration of interests:
None of the authors had competing financial or non-financial interests.

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Figure legend

Figure 1. Phylogenetic of AY.127 SARS-CoV-2 virus sequences detected in humans and hamsters. Viral genomes (Case number and detection date) detected from the studied local AY.127 human and hamster cases are highlighted in red and green, respectively. Representative AY.127 genomes from imported cases in Hong Kong (blue: internal case number and detection date) and overseas cases, and representative genomes from other pangolin lineages are included in the analysis. Only values for highly supported branches (First value: SH-aLRT ≥ 80% and Second value: UFboot ≥ 95%) are shown. Scale bar indicates estimated genetic distance.

Table 1: Chronology of outbreak investigation

Date	Event
22 December 2021	**Arrival of pet animals to warehouse, with no Syrian hamster in this shipment**
4 January 2022	Patients 2 (Mother) and Patient 4 (Daughter) visited pet shop A
7 January 2022	**Arrival of 1,009 pet animals to warehouse, with 118 Syrian hamsters in this shipment.**
	Some of these imported Syrian hamsters in the warehouse were transferred to different pet shops belonging to the same retail chain
8 January 2022	Patients 2 (Mother) and Patient 4 (Daughter) visited pet shop A
11 January 2022	Patient 1 (Pet shop A worker) experienced first symptoms
12 January 2022	Patient 2 experienced first symptoms
15 January 2022	Patient 1 tested RT-PCR positive for COVID-19
16 January 2022	Patient 1 formally registered as a positive case by a second confirmatory RT-PCR test
17 January 2022	Patient 2 tested RT-PCR positive for COVID-19
	Patient 3 (Father) experienced first symptoms
	Screening investigation at the pet shop A
18 January 2022	Patient 2 formally registered as a positive case by a second confirmatory RT-PCR test
	Patient 3 tested RT-PCR positive for COVID-19
	Screening investigation at the warehouse
	Follow-up investigation at pet shop A
19 January 2022	Patient 3 formally registered as a positive case by a second confirmatory RT-PCR test
	Patients 4 and 5 (Son) remained asymptomatic, but tested RT-PCR positive for COVID-19
	Follow-up investigation at the warehouse
	Screening investigations at pet shops B to F
	Hong Kong government ordered mass recall and culling of hamsters
20 January 2022	**Pet shop C with 2 hamsters tested positive for COVID-19**
21 January 2022	Patients 4 and 5 formally registered as positive cases by a second confirmatory PCR test

Table 2: RT-PCR confirmed samples collected in the studied sites.

Location	No. of tested samples (No. of +ve)					
	Hamster	Rabbit	Guinea pig	Chinchilla	Mouse	Total
Pet Shop	69 (7)	42 (0)	14 (0)	0	0	125 (7)
Warehouse	137 (1)	204 (0)	52 (0)	116 (0)	2 (0)	511 (1)

Table 3. Detection of SARS-CoV-2 exposed/ infected hamsters at the CWB pet shop or at the warehouse

Location	Breed	Detection frequency by individual animals (positive rate, %)					Detection frequency by cage (positive rate, %)	
		Animals sampled	Positive by sVNT	Confirmed PCR positive [a]	Inconclusive PCR positive[b]	Positive by sVNT or PCR[a]	Cages sampled	Positive cages[c]
Pet shop	**Syrian**	16	5 (31.3%)	7 (43.8%)	2 (12.5%)	8 (50.0%)	3	2 (66.7%)
	Dwarf	20	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6	0 (0%)
Warehouse	**Syrian**	12	7 (58.3%)	2 (16.7%)	2 (16.7%)	7 (58.3%)	7	5 (71.4%)
	Dwarf	55	0 (0%)	0 (0%)	3 (5.5%)	0 (0%)	20	0 (0%)

[a] Quantitative RT-PCR positive for SARS-CoV-2 N and Orf1a gene.
[b] Quantitative RT-PCR positive for SARS-CoV-2 N gene alone
[c] Cages with animals tested positive by sVNT or by quantitative RT-PCR for both N and Orf1a genes.

Table 4. Non-silent mutations found in AY.127 from infected humans and hamsters.

				Reference AY.127 Seqs				Patient #			Hamster #											
		Nt	AA								Pet shop A											Warehouse
Position	Gene	mutation	mutation	1	2	3	4	1	2	3	1	2	3	4	5	6	7	8	9	10	11	1
21614	S	C21614T	L18F	N	N	N	N	Y	Y	Y	Y	Y	-	Y	Y	Y	Y	Y	Y	Y	Y	Y
21707	S	C21707T	H49Y	N	N	N	N	Y	N	N	Y	N	-	Y	N	N	N	N	N	Y	Y	N
22842	S	A22842G	D427G	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
29670	ORF10	C29670T	T38I	N	N	N	N	N	Y	Y	N	Y	N	N	N	Y	Y	Y	Y	N	N	Y

* Unique mutations reproducibility found in both studied human and hamster cases;

**Studied European AY.127 sequences:

1) hCoV-19/Czech Republic/FNHK-Ps-002079/2021|2021-12-23|2022-01-06
2) hCoV-19/Czech Republic/UMTM367538/2021|2021-12-09|2022-01-13
3) hCoV-19/Czech Republic/NRL_16717/2021|2021-11-11|2022-01-14
4) hCoV-19/Czech Republic/FNHK-Ps-001677/2021|2021-12-08|2021-12-30