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Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study

Hui-Ling Yen, Thomas H C Sit*, Christopher J Brackman, Shirley S Y Chuk, Haogao Gu, Karina W S Tam, Pierra Y T Law, Gabriel M Leung, Malik Peiris, Leo L M Poon, the HKU-SPH study team†*

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*Contributed equally

†HKU-SPH study team members are listed at the end of the Article

School of Public Health (H-L Yen PhD, H Gu PhD, Prof G M Leung MD, Prof M Peiris FRCP, Prof L L M Poon DPhil), **WHO Collaborating Centre for Infectious Disease Epidemiology and Control** (Prof G M Leung), **and HKU-Pasteur Research Pole** (Prof M Peiris, Prof L L M Poon), **Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China; Agriculture, Fisheries and Conservation Department, Government of the Hong Kong Special Administrative Region, Hong Kong Special Administrative Region, China** (T H C Sit BVSc, C J Brackman BVSc, S S Y Chuk BVSc, K W S Tam DVM, P Y T Law PhD)**; Laboratory of Data Discovery for Health** (Prof G M Leung) **and Centre for Immunology and Infection** (Prof M Peiris, Prof L L M Poon), **Hong Kong Science Park, Shatin, Hong Kong Special Administrative Region, China**

Correspondence to: Prof Leo L M Poon, School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, China
llmpoon@hku.hk

Summary

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 evidence that these infected animals can infect humans, resulting in sustained human-to-human transmission. Following a confirmed SARS-CoV-2 infection of a pet shop worker, animals in the shop and the warehouse supplying it were tested for evidence of SARS-CoV-2 infection.

Methods In this case study, viral swabs and blood samples were collected from animals in a pet shop and its corresponding warehouse in Hong Kong. Nasal swab or saliva samples from human COVID-19 patients epidemiologically linked to the pet shop and from subsequent local cases confirmed to be infected by SARS-CoV-2 delta variant were collected. Oral swabs were tested by quantitative RT-PCR (RT-qPCR) for SARS-CoV-2 and blood samples were serologically tested by a surrogate virus neutralisation test and plaque reduction neutralisation test. The SARS-CoV-2 RT-qPCR positive samples were sequenced by next generation viral full genome sequencing using the ISeq sequencing platform (Illumina), and the viral genomes were phylogenetically analysed.

Findings Eight (50%) of 16 individually tested Syrian hamsters in the pet shop and seven (58%) of 12 Syrian hamsters in the corresponding warehouse were positive for SARS-CoV-2 infection in RT-qPCR or serological tests. None of the dwarf hamsters (n=75), rabbits (n=246), guinea pigs (n=66), chinchillas (n=116), and mice (n=2) were confirmed positive for SARS-CoV-2 in RT-qPCR tests. SARS-CoV-2 viral genomes deduced from human and hamster cases in this incident all belong to the delta variant of concern (AY.127) that had not been circulating locally before this outbreak. The viral genomes obtained from hamsters were phylogenetically related with some sequence heterogeneity. Phylogenetic dating suggests infection in these hamsters occurred around Oct 14, 2021 (95% CI Sept 15 to Nov 9, 2021). Multiple zoonotic transmission events to humans were detected, leading to onward human-to-human transmission.

Interpretation Pet hamsters can be naturally infected with SARS-CoV-2. The virus can circulate among hamsters and lead to human infections. Both genetic and epidemiological results strongly suggest that there was more than one hamster-to-human transmission event in this study. This incident also led to onward human transmission. Importation of SARS-CoV-2-infected hamsters was a likely source of this outbreak.

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Introduction

SARS-CoV-2 and its descendent variants have a wide host range besides humans. Natural human-to-animal infections have been documented in companion (dogs, cats, ferrets),[1–3] zoo (feline species[4] and gorillas), farmed (mink),[5] and wild (white-tailed deer)[6] animals. Experimental challenge has identified that non-human primates, hamsters, ferrets, American minks, cats, dogs, raccoon dogs, North American deer mice, Egyptian fruit bats, Asian small clawed otters, and white-tailed deer were highly susceptible to SARS-CoV-2 infection.[7,8] Animal-to-animal transmission has been observed in hamsters,[9] ferrets,[10] cats,[11] mink,[5] raccoon dogs,[12] fruit bats,[13] deer mice,[14] and white-tailed deer.[6] Sustained transmission and continuous evolution of SARS-CoV-2 in animals have been documented in large mink farm outbreaks[5,15] and in white-tailed deer populations.[6] So far, zoonotic transmission has only been shown for the mink-adapted SARS-CoV-2 variant during mink farm outbreaks in countries where large numbers of infected animals were housed in high density.[5,15]

SARS-CoV-2 might 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 shown transmission potential by direct contact (hamsters, ferrets, cats, raccoon dogs, and

Research in context

Evidence before this study

We searched PubMed on Jan 24, 2022, with no starting date limitations, using the terms "SARS-CoV-2" and "zoonotic transmission" for articles in English. Transmission of SARS-CoV-2 from humans to different mammalian species, including pet animals, has 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 naturally acquire SARS-CoV-2 infection and can transmit the virus back to humans. SARS-CoV-2 circulating in hamsters can lead to sustainable virus transmission in humans. Our work highlights that some pet animals can be an additional reservoir of SARS-CoV-2. This study also suggests that the pet animal trade might 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 animal reservoirs of SARS-CoV-2 in nature. Awareness and appropriate quarantine and control policies are needed to reduce these reverse zoonotic (human to animal) and zoonotic (animal to human) events.

deer mice), by fomites (hamsters) or by aerosol (hamsters, ferrets, and cats). Transmission in Syrian hamsters was more efficiently mediated via aerosols than via fomites.[9] Despite their high susceptibility to SARS-CoV-2, hamsters have not yet been reported to be infected outside of experimental settings.

Hong Kong has pursued a zero-COVID strategy and has kept transmission at very low levels,[16] with no known local circulation of SARS-CoV-2 between Oct 9 and Dec 31, 2021. On Dec 24, 2021, the omicron variant was introduced via returning air crew, which led to multiple chains of local transmission. There were no known locally acquired infections with the delta variant since Oct 9, 2021. In this Article, we report an outbreak of SARS-CoV-2 delta variant first identified in a pet shop worker on Jan 15, 2022. Subsequent investigation identified imported pet hamsters as the viral source. Such virus introduction led to more than one hamster-to-human zoonotic transmission event, resulting in onward human-to-human transmission in Hong Kong.

Methods

Sample collection

In this case study, for initial screening, oral and faecal swabs were randomly collected from different animal species housed in different cages in the involved pet shop and the supplying warehouse. For the follow-up investigations, paired blood and oral swabs were taken from hamsters in these settings. Swabs were kept chilled and blood samples were kept at room temperature until arrival at the laboratory. Blood samples were centrifuged and harvested serum was stored at 4°C until testing. Animal samples were collected for clinical diagnosis during a public health investigation led by the Government Agriculture, Fisheries and Conservation Department (AFCD). Full description of the method can be found in the appendix (p 2). Nasal swab or saliva samples from human COVID-19 patients epidemiologically linked to the pet shop and subsequent local cases confirmed to be infected by SARS-CoV-2 delta variant were collected and tested by quantitative RT-PCR (RT-qPCR) as part of routine clinical care provided by the Hong Kong Government and the viruses were genetically sequenced as part of the routine public health response (institutional review board approval: UW20-168).

RT-qPCR test

RT-qPCR positive samples collected from the initial screening were tested and independently confirmed by two laboratories (AFCD and School of Public Health, The University of Hong Kong). Samples that were positive in both assays (*ORF1b* and *N* genes of SARS-CoV-2) were classified as confirmed positives, whereas samples that were positive only in one of these assays were classified as an inconclusive result. RT-qPCR tests were conducted as described elsewhere.[17] Full description of the method can be found in the appendix (p 2).

Next generation sequencing

Representative SARS-CoV-2 positive RNA samples with adequate viral load were sequenced by next generation viral full genome sequencing using the ISeq sequencing platform (Illumina) and adapted as previously described.[3,18] The sequencing reads were trimmed and mapped to a reference virus genome (Genbank accession: MN908947.3) by BWA-MEM2. Full description of the methods and the GISAID accession numbers of deduced genomes can be found in the appendix (p 2). The viral lineage was defined by the Pango nomenclature.[19]

Phylogenetic analysis

The deduced viral genomes were analysed together with a set of representative sequences as described in the appendix (pp 2–3). The maximum likelihood phylogenies were estimated using IQ-TREE (v.2.1.3),[20] employing the TIM2+F+I nucleotide substitution model (best-fit model searched by IQ-TREE) with Wuhan-Hu-1 genome (GenBank: MN908947.3) as the

See Online for appendix

	Animal	Human
Dec 22, 2021	Arrival of pet animals to warehouse, with no Syrian hamsters in this shipment	..
Jan 4, 2022	..	Patient 2 (mother) and patient 4 (daughter) visited pet shop A
Jan 7, 2022	Arrival of 1009 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	..
Jan 8, 2022	..	Patient 2 (mother) and patient 4 (daughter) visited pet shop A
Jan 11, 2022	..	Patient 1 (pet shop A worker) had first symptoms
Jan 12, 2022	..	Patient 2 (mother) had first symptoms
Jan 15, 2022	..	Patient 1 (pet shop A worker) tested positive for SARS-CoV-2 by RT-qPCR
Jan 17, 2022	Screening investigation at pet shop A; screening investigation at the warehouse	Patient 2 (mother) tested positive for SARS-CoV-2 by RT-qPCR; patient 3 (father) had first symptoms
Jan 18, 2022	Follow-up investigation at pet shop A	Patient 3 (father) tested positive for SARS-CoV-2 by RT-qPCR
Jan 19, 2022	Follow-up investigation at the warehouse; screening investigations at pet shops B to F; Hong Kong Government ordered mass recall and culling of hamsters	Patients 4 (daughter) and 5 (son) remained asymptomatic, but tested positive for SARS-CoV-2 by RT-qPCR
Jan 20, 2022	Pet shop C with 2 hamsters tested positive for COVID-19	..

RT-qPCR=quantitative RT-PCR.

Table 1: **Chronology of outbreak investigation**

outgroup. Dating of the tree was performed by using IQ-TREE LSD2. 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[21] and Shimodaira-Hasegawa approximate likelihood-ratio test[22] were performed to evaluate the support of tree branches.

Mutation analysis

Single nucleotide polymorphisms among the studied consensus sequences were compared to the reference genome (Genbank accession: MN908947.3) using ucsc-faToVcf[23] and annotated by SnpEff.[24] The occurrences of single nucleotide variants were counted via covSPECTRUM.

For more on **covSPECTRUM** see https://cov-spectrum.org/

Surrogate virus neutralisation test (svNT)

Serum samples from hamsters were tested for SARS-CoV-2 antibodies with svNT (GenScript, NJ, USA), as described in the appendix (p 3). svNT has been validated for use in animals including hamsters.[25]

Plaque reduction neutralisation tests (PRNT)

The titre of neutralising antibody in hamster serum was quantified using PRNT. Hamster serum samples were incubated with 30–40 plaque-forming units of SARS-CoV-2 delta variant (hCoV-19/Hong Kong/21TM280310_HKUVOC0013/2021) and added to Vero E6 overexpressing TMRESS2 cells, as described in the appendix (p 3). The highest serum dilution that resulted in >50% reduction in the number of virus plaques was defined as the 50% plaque reduction neutralisation test ($PRNT_{50}$) antibody titre.

Virus isolation

Swabs with high viral load were inoculated onto Vero E6 cells overexpressing TMPRSS2. The cells were observed daily for cytopathic effect and suspected virus isolates confirmed by RT-qPCR. Full methods are described in the appendix (p 3).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Key events concerning the outbreak are shown in table 1. A 23-year-old female pet shop worker (patient 1), previously vaccinated with two doses of BNT162b2 (Pfizer–BioNtech; date of second dose: Sept 16, 2021), presented with sore throat and cough on Jan 11, 2022. She tested positive for SARS-CoV-2 infection by RT-qPCR on Jan 15, 2022 (cycle threshold value: 21) and was confirmed to have COVID-19 on Jan 16, 2022, by a second confirmatory RT-qPCR test. Full genome sequencing analysis revealed that the infection was caused by SARS-CoV-2 delta variant of concern (AY.127 virus lineage; figure). 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 Jan 8, 2022, where they met the index case (patient 1) and discussed matters relating to a pet hamster previously purchased by the daughter (patient 4) on Jan 4, 2022. The mother developed upper respiratory symptoms on Jan 12, 2022, tested positive for SARS-CoV-2 infection by RT-qPCR on Jan 17, 2022, and infection was confirmed by a second RT-qPCR test on Jan 18, 2022. Subsequently her husband (patient 3), daughter (patient 4), and son (patient 5) were also confirmed to be positive for SARS-CoV-2 infection via RT-qPCR (table 1). All these infected individuals were previously vaccinated with two doses. The mother's second dose of CoronaVac (Sinovac Biotech) was on Sept 24, 2021; the father's second dose of CoronaVac (Sinovac Biotech) was on Aug 14, 2021; the son's second dose of BBIBP-CorV (Sinopharm) was on June 12, 2021, and the daughter's second dose of BNT162b2 (Pfizer–BioNtech) was on July 16, 2021. The hamster purchased by them on Jan 4, 2022, was quarantined on Jan 18, 2022, and tested negative for SARS-CoV-2 infection with an RT-qPCR on Jan 20, 2022.

During the initial screening investigation of the animals at pet shop A carried out on Jan 17, 2022, 125 swab specimens were collected from hamsters (n=69), rabbits (n=42), and guinea pigs (n=14). Seven (10%) of the 69 swabs from hamsters (species unspecified), but none of those from other animals were confirmed positive for SARS-CoV-2 infection by RT-qPCR (table 2). The wholesale warehouse supplying this pet-shop chain was investigated on Jan 17, 2022, with 511 swabs collected from hamsters (n=137), rabbits (n=204), guinea pigs (n=52), chinchillas (n=116), and mice (n=2) housed there (table 2). One Syrian hamster swab was positive for SARS-CoV-2 infection by RT-qPCR.

Because 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 was done on Jan 18, 2022, and of the warehouse on Jan 19, 2022, with swabs and serum samples being collected from the Syrian and dwarf hamsters at both locations (table 3). At the pet shop, seven (44%) of 16 Syrian hamsters were confirmed to be positive for SARS-CoV-2 infection by RT-qPCR with both screening and confirmatory tests, while a further two Syrian hamsters were indeterminate for SARS-CoV-2 infection by RT-qPCR with only the screening RT-qPCR assay being positive but the confirmatory assay being negative. Five (31%) of 16 Syrian hamster serum samples were positive for SARS-CoV-2 antibodies by sVNT and confirmed by $PRNT_{50}$ with antibody titres ranging from 1:40 to 1:320 or more. Overall, eight (50%) of 16 Syrian hamsters had evidence of infection, either by serology or confirmed RT-qPCR, with four animals tested positive by both serology and RT-qPCR, three animals tested positive by RT-qPCR alone (cycle threshold values for *N* gene: 23·30, 30·38, and 37·43), and one animal tested positive by serology alone. A total of three cages housing Syrian hamsters were sampled, and two (67%) of these cages had animals with confirmed RT-qPCR or serology results (appendix p 5). By contrast, none of 20 cages housing dwarf hamsters were positive in either RT-qPCR or antibody assays. Because neutralising antibodies were readily detectable from hamsters as early as 5 days post-inoculation,[26] the detection of two animals with viral RNA but without antibodies suggests that infection might be a recent event.

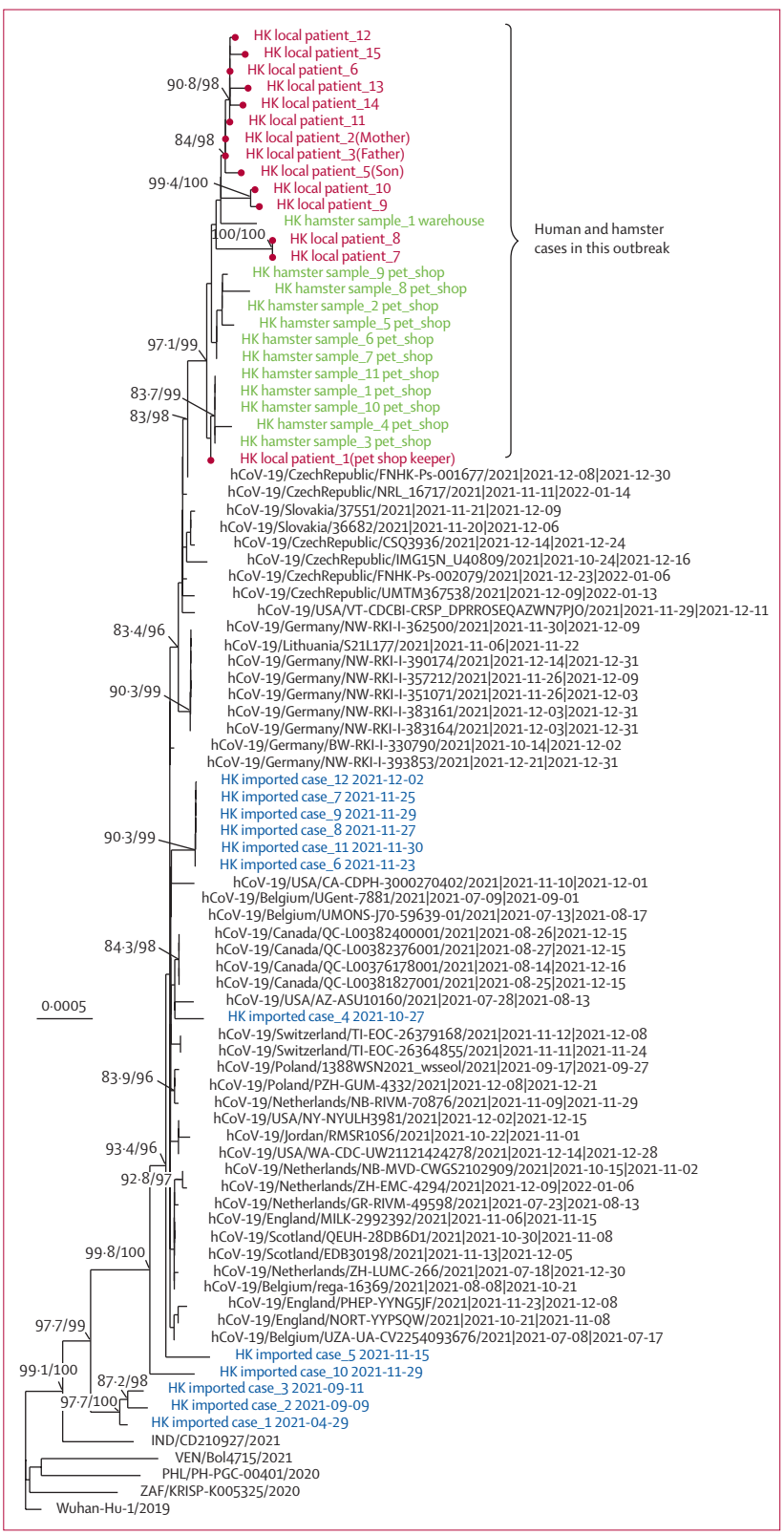

Figure: **Phylogenetic of SARS-CoV-2 delta variant AY.127 virus sequences detected in humans and hamsters**

Viral genomes (case number and detection date) detected from the studied local AY.127 human (red) and hamster (green) cases. 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 is the Shimodaira-Hasegawa approximate likelihood ratio ≥80% and second value is ultrafast bootstrap ≥95%) are shown. Scale bar indicates estimated genetic distance. HK=Hong Kong.

	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)

Number of tested samples (number of samples positive for SARS-CoV-2). RT-qPCR=quantitative RT-PCR.

Table 2: **SARS-CoV-2 RT-qPCR confirmed samples collected in the studied sites**

	Detection frequency by individual animals, positive rate (%)					Detection frequency by cage, positive rate (%)	
	Animals sampled	Positive by sVNT	Confirmed PCR positive*	Inconclusive PCR positive†	Positive by sVNT or PCR*	Cages sampled	Positive cages‡
Pet shop							
Syrian hamster	16	5 (31%)	7 (44%)	2 (13%)	8 (50%)	3	2 (67%)
Dwarf hamster	20	0	0	0	0	6	0
Warehouse							
Syrian hamster	12	7 (58%)	2 (17%)	2 (17%)	7 (58%)	7	5 (71%)
Dwarf hamster	55	0	0	3 (5%)	0	20	0

Data are n or n (%). sVNT=surrogate virus neutralisation test. *Quantitative RT-PCR positive for SARS-CoV-2 *N* and *Orf1a* genes. †Quantitative RT-PCR positive for SARS-CoV-2 *N* gene alone ‡Cages with animals tested positive by sVNT or by quantitative RT-PCR for both *N* and *Orf1a* genes.

Table 3: **Detection of SARS-CoV-2 exposed or infected hamsters at the pet shop or at the warehouse**

12 Syrian hamsters (from seven cages) and 55 dwarf hamsters (from 20 cages), were sampled at the warehouse on Jan 19, 2022 (table 3). Two (17%) of the swabs were positive for SARS-CoV-2 by RT-qPCR (cycle threshold values for *N* gene: 29·14 and 38·74) and seven (58%) of the serum samples had evidence of antibody by svNT and confirmed by $PRNT_{50}$ with antibody titres ranging from 1:40 to 1:320 or more. Seven (58%) of 12 Syrian hamsters had evidence of confirmed RT-qPCR or serologically confirmed SARS-CoV-2 infection, with two animals tested positive by both serology and RT-qPCR and five 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 (Yen H, unpublished). Although viral kinetics in oral swabs has not been determined, the detection of five animals with antibodies but without viral RNA suggest that infection might have occurred earlier. Among the seven cages housing Syrian hamsters, five (71%) cages had infected animals (appendix p 5). None of 55 dwarf hamsters from 20 cages sampled were positive for SARS-CoV-2 in the confirmatory RT-qPCR or serological test.

Hamster swabs positive for SARS-CoV-2 by RT-qPCR with high viral load (cycle threshold values of <30) were cultured for virus isolation and two virus isolates were obtained, one from the warehouse and one from pet shop A.

There was no evidence of overt illness in the hamsters sampled in pet shop A or the warehouse. Because the warehouse supplied animals to other retail outlets in Hong Kong, five additional pet shops (B to F) were sampled on Jan 19, 2022 (appendix p 6). Two of the 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 the Netherlands to Hong Kong in two different batches (arrival dates Dec 22, 2021, and Jan 7, 2022). The consignment that arrived on Dec 22, 2021, was transported by Qatar Airways and transited in Doha, Qatar, involving change of aircraft; the transit time was around 15 h. Water was topped up, but no food was provided to the animals. This consignment had 96 rabbits, 990 *Phodopus sungorus* (white dwarf hamster), and 90 *Phodopus roborovskii* (Roborovski dwarf hamster). The consignment that arrived on Jan 7, 2022, was transported by KLM, which stopped over in Bangkok, Thailand, 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 white dwarf hamsters, 118 Syrian hamsters, 25 guinea pigs, and 30 chinchillas. The hamsters were initially kept in the warehouse on arrival and smaller consignments delivered to the retail shops, meaning that the warehouse did not operate on an all-in all-out basis. Some hamsters arriving on Jan 7, 2022, were transferred to pet shop A on the day of arrival.

At the time of preparing this Article, additional human cases with an epidemiological link to hamsters or hamster-related human cases were detected. As of Feb 3, 2022, there were 82 patients in this hamster-related cluster, all of whom were confirmed positive for SARS-CoV-2 infection by RT-qPCR and tested positive for the Leu452Arg mutation in the spike protein (delta).

Specimens from the early human cases (n=14), including patients 1,2, 3, and 5, positive hamster samples collected in pet shop A (n=11), and the warehouse (n=1) had full viral genome sequence analysis. The deduced viral genomes all belonged to the delta AY.127 viral lineage. These sequences were clustered together in the tree (figure), indicating that these viruses were genetically closely related.

The deduced sequences from these human and hamster cases were highly similar, but not identical. Using the sequences deduced from the first two reported human cases as examples, the deduced sequences differed from those from hamsters by 1 to 13 nucleotides (appendix p 7). The divergent date of this cluster of human and hamster viruses was estimated to be on Oct 14, 2021 (95% CI Sept 15 to Nov 9, 2021; appendix p 4). The viral genome isolated from patient 1 was phylogenetically distinct (five nucleotides different) from those of patients 2 and 3, which were identical (figure). However, some virus sequences from hamsters in pet shop A (samples 1 and 10) only differ by one nucleotide

	Amino acid mutation*	Reference AY.127 genome sequences				Patient			Hamster										
		1†	2‡	3§	4¶	Shop keeper	Mother	Father	Pet shop A										Warehouse
									1‖	2	3	4	5	6	7**	8	9	10	11	1
***S* gene**																				
21614C→T	Leu18Phe	No	No	No	No	Yes	Yes	Yes	Yes	Yes	..	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21707C→T	His49Tyr	No	No	No	No	Yes	No	No	Yes	No	..	Yes	No	No	No	No	No	Yes	Yes	No
22842A→G	Asp427Gly	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
***ORF10* gene**																				
29670C→T	Thr38Ile	No	No	No	No	No	Yes	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes

*Unique non-silent mutations reproducibility found in both studied human and hamster cases. †European AY.127 sequence hCoV 19/Czech Republic/FNHK-Ps-002079/2021|2021-12-23|2022-01-06. ‡European AY.127 sequence hCoV-19/Czech Republic/UMTM367538/2021|2021-12-09|2022-01-13. § European AY.127 sequence hCoV-19/Czech Republic/NRL_16717/2021|2021-11-11|2022-01-14. ¶European AY.127 sequence hCoV-19/Czech Republic/FNHK-Ps 001677/2021|2021-12-08|2021-12-30. ||Representative viral genome that is genetically closest to the one detected in patient 1 (pet shop keeper).**Representative viral genome that is genetically closest to the one detected in patients 2 (mother) and 3 (father).

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

from those of patient 1. Patients 2 and 3 had viruses with genetic sequence closer (three nucleotide difference) to hamster sample 7 in pet shop A. These genetic and phylogenetic results highly suggest that patient 1 and patient 2 independently acquired the infection from hamsters at the pet shop rather than having been infected by each other. In addition, patients 7–10 (reported date Jan 20 to Jan 22, 2022) had viral sequences that were phylogenetically slightly different from the other patients. These patients had an epidemiological link to hamsters, suggesting that there might have been additional zoonotic transmission events of SARS-CoV-2 from hamsters to humans.

Patient 3 did not visit the pet shop. Because the hamster purchased by this family was negative for SARS-CoV-2 by RT-qPCR, it was more likely that patient 3 acquired the infection from his spouse (patient 2). These findings also indicate that the SARS-CoV-2 virus circulating in hamsters can transmit between humans. This was further supported by the phylogenetic results of patients 6 and patients 11–15 (figure). These patients had no epidemiological link to hamsters, but they all had an epidemiological link to the family or its related cases.

The virus sequences in hamsters were 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 travellers in Hong Kong were 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 via imported hamsters from Europe. Using some recent and genetically closely related European AY.127 viral sequences from humans as references, there were four unique non-silent mutations that can be reproducibly found in both studied human and hamster infections in Hong Kong (table 4; appendix p 8). Three of these mutations were in the spike viral protein, with two mutations in the N-terminal domain (NTD; Leu18Phe and His49Tyr) and one mutation in the receptor binding domain (Asp427Gly) in the S1 region. The Leu18Phe mutation can affect the binding of some NTD-specific antibodies[27] and the His49Tyr mutation can enhance viral entry.[28] Asp427Gly is not located in the receptor binding motif that direct interacts with host ACE2,[29] and its impact on ACE2 receptor binding and other biological functions require further investigation. These three spike mutations can be found in other sequences submitted to GISAID at various frequencies (Leu18Phe 3·31%; His49Tyr 0·17%, and Asp427Gly 0·01%). Whether these three mutations found in the hamster viruses were pre-existing or adaptive mutations requires further investigation.

Discussion

Our findings provide evidence of maintenance of SARS-CoV-2 delta variant (AY.127) by hamster-to-hamster transmission between pet Syrian hamsters, hamster-to-human zoonotic transmission, and further onward spread between humans.

Specifically, we found that Syrian hamsters at a warehouse and two pet shops (A and C) supplied by the same warehouse had evidence of SARS-CoV-2 infection. The viruses in hamsters in these premises were 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 evolutionary rate of SARS-CoV-2 in hamsters might differ from that in humans and requires further investigation. Nonetheless, 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 viruses in 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 independent hamster-to-human

zoonotic transmission events in this study. Given that viruses in hamsters was similar to the virus sequenced from the warehouse, and because 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 Jan 4, 2022, and again on Jan 8, 2022. The hamster purchased by these two patients on Jan 4, 2022, was negative for SARS-CoV-2 by RT-qPCR. Because patient 2 developed symptoms on Jan 12, 2022, and given the mean incubation period of SARS-CoV-2 is around 5 days, it would be probable that she acquired infection from infected hamsters during her visit to the pet shop on Jan 8, 2022, rather than the previously purchased hamster. The alternative hypothesis that the index case (patient 1) 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 were 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. SARS-CoV-2 delta variant was not known to be in circulation in Hong Kong for 3 months before this outbreak. None of the previously known locally acquired delta variant infections belonged to the AY.127 viral lineage. All known AY.127 cases detected in Hong Kong before this outbreak only involved incoming travellers, detected at the airport or in quarantine, with the last AY.127 case being detected in a quarantined traveller on Dec 13, 2021. Importation of SARS-CoV-2-infected hamsters was therefore a likely source of introduction of this chain of infection into Hong Kong. Although the omicron variant is increasingly becoming the dominant virus lineage in many parts of the world, delta AY.127 lineage continued to be found in parts of Europe.[30] There were two shipments arriving at the warehouse, but the shipment on Dec 22, 2021, had only dwarf hamsters and the shipment on Jan 7, 2022, had only Syrian hamsters. Thus, the Jan 7, 2022 shipment was a probable source of SARS-CoV-2 delta AY.127 introduction. It was established that hamsters arriving on this shipment to the warehouse were supplied the same day to pet shop A. This further corroborated the animal-to-human transmission risk at pet shop A.

This study has some limitations. Not all the hamsters in the concerned pet shops and warehouse were studied. Imported hamsters sold before this investigation cannot be tested. Information about the pet trading practices and these animal facilities is scarce. Thus, this study might underappreciate the virus diversity found in the affected hamster population. Although unlikely, the possibility of an undetected local chain of transmission of SARS-CoV-2 delta AY.127 leading to infection of hamsters in the warehouse cannot be excluded.

Spillover events 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 farms with a high number and density of animals. There have been reports of zoonotic transmission of mink adapted SARS-CoV-2 to humans in mink farms in Europe.[5,15] Pet dogs and cats have been reported to acquire SARS-CoV-2 infection from infected humans within the household but there is no evidence of transmission of virus back to humans.[3,31] This case report is 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 that circulated in hamsters, which is still genetically highly similar to human SARS-CoV-2, can lead to human-to-human transmission. This incident demonstrates that SARS-CoV-2 can be 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.[32]

Multiple reports, including this one, have suggested the ease with which SARS-CoV-2 can spill-back from humans to pets (eg, dogs, cats, hamsters), farmed animals (eg, mink), and wildlife (eg, white-tailed deer). Although many of these spillovers do not result in maintenance of the virus in the animal species, it has been shown to occur in mink, white-tailed deer, and hamsters. Because surveillance at the animal–human interface is so sparse, it is probable that these examples are part of a wider problem. Such events provide opportunity for the virus to evolve in unsuspected and in unpredictable ways, with possibility of future zoonotic transmission events leading to novel variants in the human population. There might also be unpredictable adverse outcomes in wildlife. Our findings, together with those from others, highlight the need of systematic surveillance of SARS-CoV-2 in both wild and domesticated animals. Mammals known to transmit SARS-CoV-2 should also be monitored on a regular basis. For human COVID-19 cases with atypical SARS-CoV-2 sequence features, additional testing on animals in affected sites and investigation into their animal contact history should be considered. The present study has also highlighted the possibility of viruses being moved across international boundaries via the pet trade.

In summary, we provide evidence of pet hamsters naturally acquiring SARS-CoV-2 delta variant and being the source of human infection. We also provide evidence suggesting the possibility of international movement of SARS-CoV-2 via the pet trade. The relatively low level of SARS-CoV-2 transmission in Hong Kong at the beginning of this outbreak and the application of the One Health approach in this investigation probably allowed the detection and investigation of this zoonotic incidence. Similar events might 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 an additional 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. Additional control measures that prevent reverse zoonosis of SARS-CoV-2 from humans to animals might help to reduce these undesirable animal-to-human transmission events.

The HKU-SPH study team members

Samuel M S Cheng, Lydia D J Chang, Pavithra Krishnan, Daisy Y M Ng, Gigi Y Z Liu, Mani M Y Hui, Sin Ying Ho, Wen Su, Sin Fun Sia, Ka-Tim Choy, Sammi S Y Cheuk, Sylvia P N Lau, Amy W Y Tang, Joe C T Koo, Louise Yung (all members are from the School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, China).

Contributors

THCS, H-LY, GML, MP, and LLMP conceptualised the study and provided supervision. SSYC (Government Agriculture, Fisheries and Conservation Department [AFCD]), KWST, WS, SFS, and K-TC facilitated or conducted field investigations; CJB, PYTL, SMSC, and HG curated the data; SSYC (The University of Hong Kong), LDJC, DYMN, PK, GYZL, MMYH, SYH, SPNL, AWYT, JCTK, and LY did the laboratory work; H-LY, GML, MP, and LLMP acquired funding; H-LY, GML, MP, and 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.

Declaration of interests

GML is an official expert adviser to the Hong Kong Government on COVID-19. All other authors declare no competing interests.

Data sharing

Viral genomes deduced from this study are deposited on GISAID (https://www.gisaid.org/) and the accession numbers for each genome are listed in the appendix (p 2). RT-qPCR and serological test results are available on request by contacting the corresponding author.

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References

1 Dileepan M, Di D, Huang Q, et al. Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. *Virulence* 2021; **12:** 1597–609.
2 Račnik J, Kočevar A, Slavec B, et al. Transmission of SARS-CoV-2 from human to domestic ferret. *Emerg Infect Dis* 2021; **27:** 2450–53.
3 Sit THC, Brackman CJ, Ip SM, et al. Infection of dogs with SARS-CoV-2. *Nature* 2020; **586:** 776–78.
4 McAloose D, Laverack M, Wang L, et al. From people to *Panthera*: natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. *MBio* 2020; **11:** e02220-20.
5 Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* 2021; **371:** 172–77.
6 Hale VL, Dennis PM, McBride DS, et al. SARS-CoV-2 infection in free-ranging white-tailed deer. *Nature* 2021; published online Dec 23. https://doi.org/10.1038/s41586-021-04353-x.
7 Murphy HL, Ly H. Understanding the prevalence of SARS-CoV-2 (COVID-19) exposure in companion, captive, wild, and farmed animals. *Virulence* 2021; **12:** 2777–86.
8 World Organisation For Animal Health. Infection with SARS-CoV-2 in animals. 2021. https://www.oie.int/app/uploads/2021/11/en-factsheet-sars-cov-2-20211025.pdf (accessed Feb 5, 2022).
9 Sia SF, Yan LM, Chin AWH, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* 2020; **583:** 834–38.
10 Kim YI, Kim SG, Kim SM, et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. *Cell Host Microbe* 2020; **27:** 704–709.e2.
11 Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science* 2020; **368:** 1016–20.
12 Freuling CM, Breithaupt A, Müller T, et al. Susceptibility of raccoon dogs for experimental SARS-CoV-2 infection. *Emerg Infect Dis* 2020; **26:** 2982–85.
13 Schlottau K, Rissmann M, Graaf A, et al. SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *Lancet Microbe* 2020; **1:** e218–25.
14 Fagre A, Lewis J, Eckley M, et al. SARS-CoV-2 infection, neuropathogenesis and transmission among deer mice: implications for spillback to New World rodents. *PLoS Pathog* 2021; **17:** e1009585.
15 Hammer AS, Quaade ML, Rasmussen TB, et al. SARS-CoV-2 transmission between mink (Neovison vison) and humans, Denmark. *Emerg Infect Dis* 2021; **27:** 547–51.
16 Gu H, Chu DKW, Chang LDJ, et al. Genetic diversity of SARS-CoV-2 among travelers arriving in Hong Kong. *Emerg Infect Dis* 2021; **27:** 2666–68.
17 Chu DKW, Pan Y, Cheng SMS, et al. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin Chem* 2020; **66:** 549–55.
18 Gu H, Krishnan P, Ng DYM, et al. Probable transmission of SARS-CoV-2 omicron variant in quarantine hotel, Hong Kong, China, November 2021. *Emerg Infect Dis* 2022; **28:** 460–62.
19 Rambaut A, Holmes EC, O'Toole Á, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* 2020; **5:** 1403–07.
20 Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020; **37:** 1530–34.
21 Minh BQ, Nguyen MA, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* 2013; **30:** 1188–95.
22 Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 2010; **59:** 307–21.
23 University of California Santa Cruz Genomics Institute. Nextstrain subset of GISAID EpiCoV TM sample mutations. http://genome.ucsc.edu/cgi-bin/hgTrackUi?db=wuhCor1&g=nextstrainSamples (accessed Jan 22, 2022).
24 Cingolani P, Platts A, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)* 2012; **6:** 80–92.
25 Perera RAPM, Ko R, Tsang OTY, et al. Evaluation of a SARS-CoV-2 surrogate virus neutralization test for detection of antibody in human, canine, cat, and hamster sera. *J Clin Microbiol* 2021; **59:** e02504-20.

26 Horiuchi S, Oishi K, Carrau L, et al. Immune memory from SARS-CoV-2 infection in hamsters provides variant-independent protection but still allows virus transmission. *Sci Immunol* 2021; **6:** eabm3131.

27 McCallum M, De Marco A, Lempp FA, et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell* 2021; **184:** 2332–2347.e16.

28 Ozono S, Zhang Y, Ode H, et al. SARS-CoV-2 D614G spike mutation increases entry efficiency with enhanced ACE2-binding affinity. *Nat Commun* 2021; **12:** 848.

29 Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020; **581:** 215–20.

30 O'Toole Á, Scher E, Rambaut A. Lineage AY.127. 2021. https://cov-lineages.org/lineage.html?lineage=AY.127 (accessed Feb 5, 2022).

31 Barrs VR, Peiris M, Tam KWS, et al. SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. *Emerg Infect Dis* 2020; **26:** 3071–74.

32 Reed KD, Melski JW, Graham MB, et al. The detection of monkeypox in humans in the western hemisphere. *N Engl J Med* 2004; **350:** 342–50.