Probable Animal-to-Human Transmission of SARS-CoV-2 Delta Variant AY.127 Causing a Pet Shop-Related COVID-19 Outbreak in Hong Kong

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Summary: We report a SARS-CoV-2 Delta variant AY.127 outbreak related to a pet store in Hong Kong. Genomic analysis showed that multiple strains were involved. Our study highlighted the possibility of SARS-CoV-2 being transmitted from hamsters to human in the community.

ABSTRACT

Background. SARS-CoV-2 can infect human and other mammals, including hamsters. Syrian (*Mesocricetus auratus*) and dwarf (*Phodopus* sp.) hamsters are susceptible to SARS-CoV-2 infection in the laboratory setting. However, pet shop-related COVID-19 outbreaks have not been reported.

Methods. We conducted an investigation of a pet shop-related COVID-19 outbreak due to Delta variant AY.127 involving at least three patients in Hong Kong. We tested samples collected from the patients, environment, and hamsters linked to this outbreak and performed whole genome sequencing analysis of the RT-PCR-positive samples.

Results. The patients included a pet shop keeper (Patient 1), a female customer of the pet shop (Patient 2), and the husband of Patient 2 (Patient 3). Investigation showed that 17.2% (5/29) and 25.5% (13/51) environmental specimens collected from the pet shop and its related warehouse, respectively, tested positive for SARS-CoV-2 RNA by RT-PCR. Among euthanized hamsters randomly collected from the storehouse, 3% (3/100) tested positive for SARS-CoV-2 RNA by RT-PCR and seropositive for anti-SARS-CoV-2 antibody by ELISA. Whole genome analysis showed that although all genomes from the outbreak belonged to the Delta variant AY.127, there were at least 3 nucleotide differences among the genomes from different patients and the hamster cages. Genomic analysis suggests that multiple strains have emerged within the hamster population, and these different strains have likely transmitted to human either via direct contact or via the environment.

Conclusions. Our study demonstrated probable hamster-to-human transmission of SARS-CoV-2. As pet trading is common around the world, this can represent a route of international spread of this pandemic virus.

Keywords: SARS-CoV-2; Delta variant AY.127; hamsters, transmission, pet**.**

INTRODUCTION

Coronaviruses are a diverse group of positive-stranded RNA viruses belonging to the family *Coronaviridae* in the order Nidovirales found in a wide range of mammals and birds [1]. Coronaviruses are known to have repeatedly crossed species barriers with some emerging as important human pathogens. SARS-CoV-1 was likely originated in bats with subsequent amplification in civets before jumping into human [2, 3]. Dromedary camels (*Camelus dromedarius*) play an important role in the transmission of Middle East respiratory syndrome coronavirus (MERS-CoV) [4]. Bats and rodents are generally considered as the animal reservoirs of alphacoronaviruses and betacoronaviruses, whereas birds are the likely reservoirs of deltacoronaviruses and gammacoronaviruses [1]. As of today, there are seven coronaviruses known to cause disease in humans.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the Coronavirus Disease 2019 (COVID-19) pandemic, was first discovered in patients in Wuhan, China, in late 2019, and was soon found to be highly transmissible in the human population [5, 6]. This pandemic virus can be transmitted via the droplet and contact routes, and airborne transmission may also be important [7, 8]. Various animal species have been reported to be infected by SARS-CoV-2, including companion animals like pet dogs and cats, zoo animals, mink on mink farms, and wild white-tailed deer [9]. The World Organization for Animal Health (OIE) reported that infection with SARS-CoV-2 has been documented in at least 17 animal species, and outbreaks have been documented in 15 animal species as of 31st December 2021 [10]. However, animal-to-human transmission has been generally considered to be rare [11]. Mink (the American mink, *Neogale vison*)-associated COVID-19 human cases have been documented in Europe in late 2020, resulting in mass culling of 17 million of minks in November 2020 [12].

Hong Kong Special Administrative Region, China, has adopted a "zero tolerance" policy for COVID-19 and there were only sporadic locally-acquired cases. Alarmingly, in January 2022, three local patient-cases of COVID-19 caused by the SARS-CoV-2 Delta variant AY.127 which has not been found in the local community in Hong Kong previously. All three patients were epidemiologically linked to either a local pet shop or to each other. Investigations showed that the environment of the pet shop as well as the warehouse supplying the hamsters to the pet shop were contaminated with SARS-CoV-2. Epidemiological and virological investigations demonstrated that the index patient was possibly infected by contact with SARS-CoV-2-infected animals or the contaminated environment in the pet shop, and then transmitted the virus to the others. Herein, we report the details of the epidemiological and virological investigations of this pet shop-related outbreak.

METHODS

Patient and environmental specimens

Archived respiratory specimens tested positive for SARS-CoV-2 by reverse transcription-polymerase chain reaction (RT-PCR) were retrieved from clinical laboratories. Environmental swabs were collected at the pet shop (at Causeway Bay District) and the warehouse (at Tai Po District) which supplied the hamsters to the pet shop. The initial RT-PCR was performed at commercial laboratories and hospital laboratories in Hong Kong, and confirmed by the Public Health Laboratory Services Branch (PHLSB) of Department of Health of the Hong Kong Special Administrative Region Government. This study was approved by the the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 13-372) and the Kowloon West Cluster Research Ethics Committee (KW/EX-21-151[165-01] and KWC-20200040).

Whole genome sequencing and bioinformatics analysis

Whole genomes sequencing of patient specimens were performed using Nanopore (Nanopore protocol - PCR tiling of COVID-19 [Version: PTC_9096_v109_revH_06Feb2020] according to the manufacturer's instructions with minor modifications (Oxford Nanopore Technologies) as we described previously [13]. Illumina sequencing was used for environmental specimens. For bioinformatics analysis, the recommended ARTIC bioinformatics workflow was used with minor modifications applied as described previously [13]. Maximum-likelihood whole genome phylogenetic tree was constructed using IQ-TREE2 [14]. (See supplementary methods for details). The genome sequences have been deposited in GISAID database (Supplementary Table S1)

Anti-SARS-CoV-2 antibody detection in hamster serum samples

Enzyme immunoassay (EIA) was performed as previously described [15, 16]. Briefly, 96-well plates (Nunc, Rochester, NY, USA) were coated with 0.1 μg/well of SARS-CoV-2 receptor-binding domain (RBD) in 100 μl of 0.05 M NaHCO3 (pH 9.6) overnight at 4°C. One hundred microliters of of heat-inactivated serum samples were serially diluted after blocking, and then they were added to the wells and incubated at 37°C for 1 h. The attached antibodies were detected using horseradish-peroxidase-conjugated rabbit anti-hamster IgG antibody (A18895 from Invitrogen, Waltham, MA, USA), followed by addition of diluted 3,3,5,5-tetramethylbenzidine single solution (Invitrogen) and 0.3 N H_2SO_4. The optical density at 450 nm (OD_{450}) was read using a microplate reader. Serum samples of a Delta-infected hamsters and mock-infected hamsters obtained from HKU CCMR which were not linked with the pet shop hamsters were included as positive and negative controls,

respectively. The positive samples were then confirmed with iFlash-2019-nCoV neutralization antibody assay (Shenzhen YHLO Biotech Co., Ltd.).

RESULTS

A cluster of epidemiologically-linked COVID-19 patients

The index patient (Patient 1) was a 23-year old female who worked as a shopkeeper in a pet store. On January 11 2022, she developed rhinorrhea, sore throat and cough (Figure 1). Four days post-symptom onset (PSO), her deep throat saliva specimen tested positive for SARS-CoV-2 by RT-PCR (Ct value: 21). She had received 2 doses of BNT162b2 vaccine on 21 August 2021 and 16 September 2021. She wore surgical mask during work. Her duties included feeding the animals, cleaning cages and the environment, and serving customers. She did not visit the warehouse housing about hamsters for distribution to the involved pet shop and other pet shops in Hong Kong. At the time of writing, none of her close contacts tested positive for SARS-CoV-2 RT-PCR.

Patient 2 was a 67-year-old female. She first developed headache on January 12, 2022 and then cough two days later. Her deep throat saliva specimen collected on day 4 PSO, which tested positive for SARS-CoV-2 by RT-PCR (Ct value: 13). She had received 2 doses of CoronaVac vaccine on 26 August 2021 and 24 September 2021. Eight days prior to symptom onset, her daughter purchased a hamster at the pet store. Four days prior to symptom onset, the patient visited the pet shop and had direct contact with Patient 1. Her daughter has been quarantined and SARS-CoV-2 has been undetectable in all respiratory specimens at the time of writing.

Patient 3 was the 73-year-old husband of Patient 2. He first developed productive cough on January 17, 2022, five day after the symptom onset of Patient 2. One day PSO, his deep throat saliva specimen tested positive for SARS-CoV-2 by RT-PCR (Ct value = 12). He

had received two doses of CoronaVac on 17 July 2021 and 14 August 2021. He did not visit the pet shop and did not have direct contact with Patient 1. At the time of writing, all three patients were clinically stable.

Virological investigations of environmental and animal specimens at the pet shop

To determine whether there was environmental contamination at the pet store, 29 environmental swab specimens were collected. Five specimens (17.2%) tested positive for SARS-CoV-2 by RT-PCR (Table 1). Among the 6 oral swabs and 2 anal swabs collected from 4 hamsters, 2 rabbits, and 2 chinchillas, none was positive for SARS-CoV-2 by RT-PCR. Serum samples were not available for antibody detection to look for past infection in these animals.

Virological investigations of environmental and animal specimens at the warehouse

Prior to transferal to the involved pet shop, the hamsters were housed in a warehouse. On December 22, 2021 and January 7, 2022, two batches of hamsters were imported to the warehouse from the Netherlands. To investigate for evidence of SARS-CoV-2 infection in the warehouse housing about 1,000 hamsters and a small number of rabbits and chinchilla distributed to the involved pet shop and other pet shops in Hong Kong, RT-PCR was performed on 51 environmental swabs. A total of 13 of these 51 (25.5%) environmental swabs mainly obtained from the animal cages were positive for SARS-CoV-2 RNA by RT-PCR (Table 1). Further investigations were then performed on 100 randomly selected euthanized dwarf hamsters housed in the warehouse, as the small number of rabbits and chinchillas had already been removed from the warehouse prior to the warehouse visit. The lung tissues of three of these 100 (3.0%) hamsters tested positive for the SARS-CoV-2 by RT-PCR with Ct values of 34-35. Among these 100 hamsters, 79 had sufficient volume of

serum specimens for antibody detection. Anti-SARS-CoV-2 antibody was detected by both the in-house developed ELISA and a commercial surrogate neutralization antibody assay in the serum samples of the same three hamsters. Overall, the relatively low viral loads and positive serum anti-SARS-CoV-2 antibody response suggested that the three hamsters had evidence of SARS-CoV-2 infection.

Genome analysis

Whole genome sequencing was performed for the respiratory specimens from Patient 1 (combined nasopharyngeal swab and throat swab), Patient 2 (combined nasopharyngeal swab and throat swab) and Patient 3 (deep throat saliva), and the environmental swabs. Genome sequencing was successful for all 3 patients and 1 swab specimens from a hamster cage (hamster cage 1). The patient and environmental swab specimens from pet shop belonged to AY.127. These were phylogenetically distinct from other AY.127 strains previously identified in Hong Kong in from November to December 2021 (Figure 2).

The genome sequences of Patients 1, 2 and 3 contain the spike protein mutations L18F and D427G when compared with the most closely related strain according to GISAID (hCoV-19/Czech Republic/FNHK-Ps-001677/2021) (Figure 2). The genome sequences of Patient 2 and Patient 3 were identical. There were 5 base pair (bp) difference between Patient 1 and Patient 2 or 3 (Table 2). The hamster cage swab 1 from the pet shop was also different from the human cases (4 bp difference from case 1, 2 or 3). Patient 1 has a unique spike mutation H49Y. Both Patient 2 and 3 and the hamster cage swab 1 contained ORF10 T38I. The hamster cage swab 1 contained a unique spike mutation A93S.

DISCUSSION

Prior to the present study, pet shop-related COVID-19 outbreak has not been reported and animal-to-human transmission has been generally considered to be rare. Herein, we reported the epidemiological and virological investigations of a pet shop-related outbreak of COVID-19 with possible interspecies transmission. Our epidemiological investigations revealed that the three vaccinated patients in this cluster were infected by a unique branch of the Delta variant AY.127. The most closely related virus strains of this SARS-CoV-2 Delta variant AY.127 was reported in Europe. The index patient did not visit the warehouse which was housing SARS-CoV-2-infected hamsters. Thus, the most likely scenario was that the index patient acquired the infection at the pet shop, either through direct contact with infected animals or the contaminated environment. Patient 2 could have acquired the infection from Patient 1, directly from the pet shop, or the hamster her daughter brought home, although this hamster was not available for testing. However, it is less likely that Patient 2 directly acquired the infection from Patient 1 as there were 5 nucleotide difference between Patient 1 and Patient 2. The genome sequences of the virus strains from Patients 2 and 3 were identical, indicating that Patient 3 has likely been infected by Patient 2 after the later had acquired the infection from the pet shop. Another remote possibility is that an undetected patient infected with this Delta variant had visited the pet shop and infected the index patient directly or contaminated the environment and/or infected the animals in the pet shop. However, given the stringent public health measures and the prior absence of similar Delta variant AY.127 in Hong Kong, this scenario was much less likely.

Virological investigations revealed that the environmental specimens collected at the pet shop (17.2%) were positive for SARS-CoV-2 RNA by RT-PCR. In the laboratory setting, both golden Syrian hamsters (*Mesocricetus auratus*) and dwarf hamsters (*Phodopus* sp.) are naturally infectable by the lineage b betacoronavirus SARS-CoV-2 as their ACE2/spike

contact residues are highly similar to those of human [17, 18]. Moreover, transmission of SARS-CoV-2 among hamsters by either contact or non-contact are highly efficient [17, 19]. The clinical manifestations of the hamsters may be mild when the exposure dose is low [20]. Hamsters usually develop a self-limiting illness and recover by 1-2 weeks after exposure to SARS-CoV-2, which allows silent transmission to other hamsters and possibly human [17]. Live infectious virus particles could be detected in the respiratory tract of SARS-CoV-2-infected hamsters for at least 4 to 7 days after virus challenge in the laboratory setting.

Genome sequencing analysis provided further insights into the interspecies transmission in this outbreak. Whole genome sequencing showed that the strains from the three patients and the environmental swabs exhibited distinct amino acid differences, suggesting that the virus might have already been circulating in some animals. The patient and hamster cage virus strains in this cluster have a unique mutation in the spike protein amino acid residue 427. Structural analysis of the ancestral virus showed that the interaction between the negatively charged amino acid residue D427 and the positively charged residue K986 may stabilize the spike protein in the closed form [21]. It remains to be determined whether D427G substitution (glycine being a neutral amino acid) may cause destabilization of the spike protein. Due to the low viral load detected in the warehouse environmental and hamster samples, whole genome sequencing was unsuccessful. Interestingly, follow-up spike gene sequencing after the present epidemiological investigation revealed that the SARS-CoV-2 strain in these dwarf hamsters was phylogenetically related to the European B.1.258 strain, suggesting that there was co-circulation of at least two SARS-CoV-2 variants among the hamsters [22]. These finding emphasized the importance of hamsters in the transmission of SARS-CoV-2.

Our study had limitations. While one environmental swab specimen from a chinchilla cage in the pet shop was positive for SARS-CoV-2 RNA by RT-PCR, we were not able to

obtain chinchilla tissues and blood for testing to exclude the possibility of infection among the chinchillas. However, only 11 hamsters but no chinchilla samples collected at the pet shop tested by the Agriculture, Fisheries and Conservation Department of the HKSAR Government were found to be SARS-CoV-2 positive, suggesting that hamster was the more likely source involved in this outbreak [23]. We also could not completely exclude the remote possibility of the hamsters having acquired the infection from an infected person during their stay in the warehouse, but this was considered unlikely as this unique branch of Delta variant AY.127 was not reported in our community prior to this outbreak. Furthermore, we were not able to determine whether the virus found in the hamster cage 1 originated from patients or from infected hamsters. In summary, our study demonstrated possible interspecies transmission of SARS-CoV-2 from infected hamsters to human. As pet trading is common around the world, this can represent a route of international spread of viruses.

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AUTHOR CONTRIBUTIONS.

J.F.-W.C., G.K.H.S., K.-Y.Y., and K.K.-W.T. had roles in the study design, data collection, data analysis, data interpretation, and writing of the manuscript. S.F., J.D.I., J.-P.C., A.W.H.C., W.M.C., S.M.U.A., C.L., B.P.-C.C., T.T.-T.Y., L.-L.C., K.K.-H.C., R.L., H.Cao, V.K.-M.P., C.C.-S.C., K.-H.L., A.R.T., O.T.-Y.T., J.M.-C.C., W.-K.T., B.H.-S.L., L.-K.L., H.W.-H.L., I.T.-F.W., J.S.-L.L., E.Y.-K.W., H.Chu, C.C.-Y.Y., V.C.-C.C., K.-H.C., H.T., D.C.L., K.H.-L.N., A.K.-W.A., and I.F.-N.H. had roles in the experiments, data collection, and/or data analysis.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

REFERENCES

1. Chan JF, To KK, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol **2013**; 21(10): 544-55.
2. Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U S A **2005**; 102(39): 14040-5.
3. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science **2003**; 302(5643): 276-8.
4. Haagmans BL, Al Dhahiry SH, Reusken CB, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis **2014**; 14(2): 140-5.
5. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet **2020**; 395(10223): 514-23.
6. To KK, Sridhar S, Chiu KH, et al. Lessons learned one year after SARS-CoV-2 emergence leading to COVID-19 pandemic. Emerg Microbes Infect **2021**: 1-89.
7. Wong SC, Au AK, Chen H, et al. Transmission of Omicron (B.1.1.529) - SARS-CoV-2 Variant of Concern in a designated quarantine hotel for travelers: a challenge of elimination strategy of COVID-19. Lancet Reg Health West Pac **2021**: 100360.
8. Cheng VC, Fung KS, Siu GK, et al. Nosocomial outbreak of COVID-19 by possible airborne transmission leading to a superspreading event. Clin Infect Dis **2021**.
9. Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science **2020**; 368(6494): 1016-20.

10. (OIE). WOfAH. SARS-CoV-2 in animals – situation report 8. Available at https://www.oie.int/app/uploads/2022/01/sars-cov-2-situation-report-8.pdf. Accessed on 20th January 2022.

11. CDC. U. Animals and COVID-19. Available at https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html. Accessed on January 21, 2022.

12. 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(6525): 172-7.

13. To KK, Li X, Lung DC, et al. False COVID-19 cases due to contamination by inactivated virus vaccine. Clin Infect Dis **2021**.

14. 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(5): 1530-4.

15. Ye ZW, Yuan S, Chan JF, et al. Beneficial effect of combinational methylprednisolone and remdesivir in hamster model of SARS-CoV-2 infection. Emerg Microbes Infect **2021**; 10(1): 291-304.

16. Yuan S, Yin X, Meng X, et al. Clofazimine broadly inhibits coronaviruses including SARS-CoV-2. Nature **2021**; 593(7859): 418-23.

17. Chan JF, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. Clin Infect Dis **2020**.

18. Trimpert J, Vladimirova D, Dietert K, et al. The Roborovski Dwarf Hamster Is A Highly Susceptible Model for a Rapid and Fatal Course of SARS-CoV-2 Infection. Cell Rep **2020**; 33(10): 108488.

19. Chan JF, Yuan S, Zhang AJ, et al. Surgical mask partition reduces the risk of non-contact transmission in a golden Syrian hamster model for Coronavirus Disease 2019 (COVID-19). Clin Infect Dis **2020**.

20. Lee AC, Zhang AJ, Chan JF, et al. Oral SARS-CoV-2 Inoculation Establishes Subclinical Respiratory Infection with Virus Shedding in Golden Syrian Hamsters. Cell Rep Med **2020**; 1(7): 100121.

21. Xiong X, Qu K, Ciazynska KA, et al. A thermostable, closed SARS-CoV-2 spike protein trimer. Nat Struct Mol Biol **2020**; 27(10): 934-41.

22. Kok KH, Wong SC, Chan WM, et al. Cocirculation of two SARS-CoV-2 variant strains within imported pet hamsters in Hong Kong. Emerg Microbes Infect **2022**: 1-39.

23. Ma C. COVID-19: 2000 Hamsters, Chinchillas, Guinea Pigs and Rabbits to be culled after 11 tested positive. Available at https://harbourtimes.com/2022/01/19/covid-19-2000-hamsters-chinchillas-guinea-pigs-and-rabbits-to-be-culled-after-11-tested-positive/. Accessed on January 21, 2022.

FIGURE LEGEND

Figure 1. Timeline of the COVID-19 cluster associated with the pet shop.

Figure 2. Whole genome phylogenetic analysis showing the relationship between COVID-19 patients within pet shop cluster (Patient 1, Patient 2, and Patient 3) and the cage swab specimen collected from the pet shop (hamster cage swab 1). The trees were constructed using maximum likelihood method. The reference genome Wuhan-Hu-1 (GenBank accession number MN908947.3) was used as the root of the tree.

Table 1. Environmental and Animal Specimens Collected at the Pet Shop and Warehouse

Location	Specimen	Species	Item	Positive / Total[a]
Pet Shop	Environmental	Hamsters	Cages	**3/12**[b]
			Water bottles	0/2
			Food plates	**1/2**
		Chinchillas	Cages	**1/2**
			Water bottle	0/1
			Food plate	0/1
		Rabbits	Cages	0/7
			Water bottle	0/1
			Food plate	0/1
		Total		**5/29 (17.2%)**
	Animals	Hamsters	Oral swabs	0/4
		Chinchillas	Oral swab	0/1
			Anal swab	0/1
		Rabbits	Oral swab	0/1
			Anal swab	0/1
		Total		0/8 (0.0%)
Warehouse	Environmental	Hamsters, chinchillas and rabbits	Cages	**13/51 (25.5%)**
	Animals	Hamsters	Lung tissues	**3/100 (3.0%)**
			Faecal swabs	0/100 (0.0%)
			Sera	**3/79 (3.8%)**

[a]For detection of SARS-CoV-2 RNA by RT-PCR except for sera which were used to testing anti-SARS-CoV-2 antibody.

[b] The Ct values are between 32-33.

Table 2. Differences in the genomes between patients and environmental swabs

Gene/Protein name	Nucleotide position	Reference SARS-CoV-2 genome (MN908947.3)	Patient 1		Patient 2		Patient 3		Hamster cage swab 1	
			Nucleotide substitution	Amino acid substitution	Nucleotide substitution	Amino acid substitution	Nucleotide substitution	Amino acid substitution	Nucleotide substitution	Amino acid substitution
Nsp3	5467	C	100% C	-	99% C, 1% T	-	98% C, 1% T	-	100%T	-
Nsp4	9430	C	98% C, 1% T	-	98% T, 1% C	-	96% T, 2% C	-	N	-
Nsp4	9886	C	99% C, 1% T	-	98% T, 1% C	-	92% T, 2% C	-	96% C, 1% T	-
Spike	21707	C	98% T, 2% C	H49Y	99% C, 1% T	-	98% C, 1% T	-	98% C, 2% T	-
Spike	21839	G	99% G, 1% A	-	98% G, 0% T	-	97% G, 0% T	-	100% T	A93S
Spike	24370	C	99% C, 1% A, 1% T	-	98% T, 1% C	-	96% T, 3% C	-	99% C, 1% T	-
orf10	29670	C	99% C, 1% T	-	92% T, 7% C	T38I	90% T, 10% C	T38I	89% T, 10% C	T38I

Figure 1

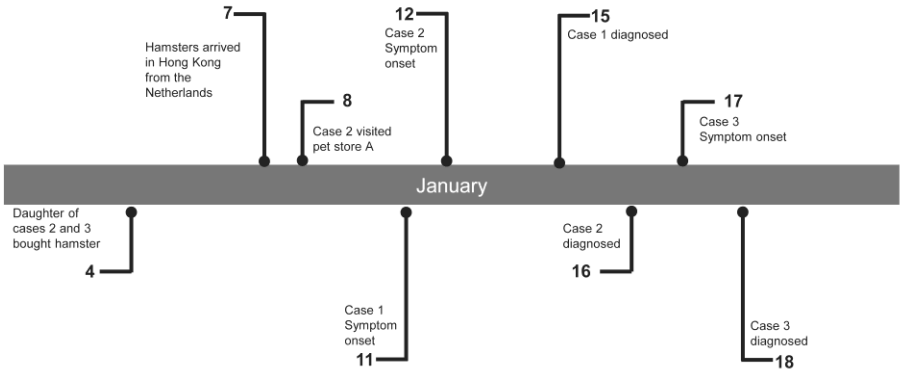
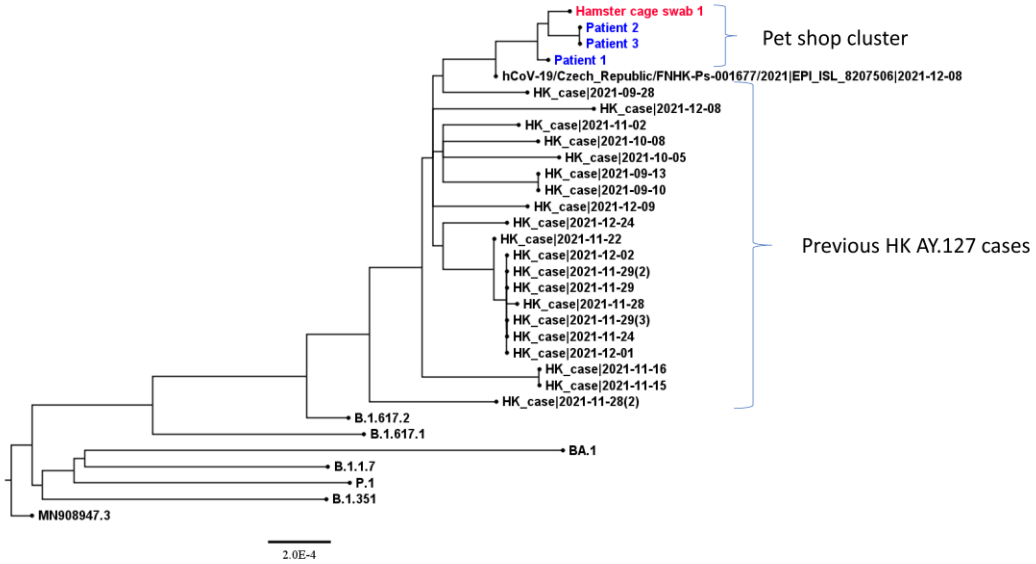


Figure 2


Hamster cage swab 1
Patient 2
Patient 3
Patient 1
hCoV-19/Czech_Republic/FNHK-Ps-001677/2021|EPI_ISL_8207506|2021-12-08
HK_case|2021-09-28
HK_case|2021-12-08
HK_case|2021-11-02
HK_case|2021-10-08
HK_case|2021-10-05
HK_case|2021-09-13
HK_case|2021-09-10
HK_case|2021-12-09
HK_case|2021-12-24
HK_case|2021-11-22
HK_case|2021-12-02
HK_case|2021-11-29(2)
HK_case|2021-11-29
HK_case|2021-11-28
HK_case|2021-11-29(3)
HK_case|2021-11-24
HK_case|2021-12-01
HK_case|2021-11-16
HK_case|2021-11-15
HK_case|2021-11-28(2)
B.1.617.2
B.1.617.1
BA.1
B.1.1.7
P.1
B.1.351
MN908947.3
2.0E-4
Pet shop cluster
Previous HK AY.127 cases