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# Surveillance for Genetic Markers of Adaptation of SARS-CoV-2 in Dogs and Cats

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## ABSTRACT

Surveillance for genetic markers of adaptation of SARS-CoV-2 in dogs and cats could help to predict the risk of variant emergence, that resulted from virus adaptation to the new host. The Delta variant of SARS-CoV-2 collected from dog and cat is of high interest, since this variant caused global major cases and fatality during the pandemic. All complete and high coverage sequence data of Delta variant of dog and cat origin, as well as samples of human origin SARS-CoV-1 were downloaded. All selected sequence data were subjected to Clustal Omega alignment. Evolutionary history was inferred by using the Maximum Likelihood method using Mega11. Species-specific markers indicating a probable species adaptation of the pandemic SARS-CoV-2 in dog and cat could not be identified. The markers found in many dog and cat samples is confirmed to be location specific markers that occurred in human origin virus.

**Key words:** SARS-CoV-2; Companion animals; Adaptation; Genetic markers

## INTRODUCTION

Role of companion animals in contributing to the transmission of the severe acute respiratory coronavirus 2 (SARS-CoV-2) is of global interest in developing a holistic approach of any pandemic control. Since being always in a close contact with the owner, transmission of the virus from humans, dog, and cat, as the two most common companion animals (Meehan et al. 2017; Shahzad et al. 2021), is important to be investigated. This zoonotic occurrence is plausible since the catalyst of the pandemic of Covid-19 was indeed a zoonotic event from an animal origin to human being (Andersen et al. 2020; Holmes et al. 2021; Pekar et al. 2021). The ancestor might have been a bat origin virus which than might have undergone gradual mutation and/or recommendation in bat, cat, or people (Mahardika 2023). Companion animals might get infected from humans and transmitted back to other humans.

Infection of SARS-CoV-2 to dog and cat caused mild to subclinical infection and the animal developed immune response to the virus. These species are indeed susceptible to SARS-CoV-2. After anthropogenic transmission (Hosie et al. 2021), i.e. from human to animal, seroprevalences can be 40-60% in dog and cat (Michelitsch et al. 2023). Risk factor for cats was number of infected humans in households, for dog was contact outside the household (Michelitsch et al. 2023). The presence and persistence of SARS-CoV-2 infection have been identified in dogs and cats from households with human COVID-19 cases in Rio de Janeiro, Brazil (Calvet et al. 2021). A study in Germany has found that the virus caused respiratory symptoms with no fatality (Michelitsch et al. 2023). Other report shows that an immune-compromised cat developing severe acute respiratory syndrome and lesions in several organs, and death (Carneiro et al. 2022). The animals could transmit the virus to other animals (Shi et al. 2020), and therefor back transmission to human being is very likely.

Accessed in January 31, 2024, data GISAID total complete and high coverage data of dog origin was 62, four was Alpha and 27 was Delta variant. Of cat origin, the total was 110, the Alpha was four and the Delta variant was 27. Complete and high coverage data of Beta, Gamma, Lambda, and Mu variants were not available. Among both variants, the Delta variant is of high interest since it caused the highest mortality peak in the course of the covid pandemic. Compared to Alpha variant, the Delta spread faster because of its capability to invade the host's immune system (Maugeri et al. 2020), has higher transmissibility (Dhawan et al. 2022; Jamil et al. 2023), and caused a sharp increase in hospitalization risk, in ICU admission, and in fatality rate (Sheikh et al. 2021).

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Here we compare all Delta variant of dog and cat origin with the representatives of Delta variant detected in human available in public database to identify probable unique adaptation markers of SARS-COV-2 in these companion animals.

## MATERIALS AND METHODS

### Ethical approval

This study did not involve life viruses and animals. The data we analyzed is purely accessible in GISAID and we have complied with GISAID data Terms of Use as expressed in the data availability.

All complete and high coverage sequence data of Delta variant of dog and cat origin, as well as all human origin dated 2023, June 2022 and 15 June 2021. We analyzed all dog and cat origin data, as well as all human origin data from Europe, Asia, and North America dated 2023 and June 2022. From sequence data of human origin dated 15 June 2021 from Europe (2991 data), Asia (280 data) and North America (552 data), we selected the first and the last 10 of the data set to cover the oldest and the most recent submitted data. All selected sequence data were separated into two files due to the limitation of online software of 4MB and subjected to Clustal Omega alignment available online (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). The files were merged and re-aligned manually using MEGA11 (Tamura et al. 2021). The first data set ID is EPI_SET_240216nc (https://doi.org/10.55876/gis8.240216nc). The second dataset EPI_SET_240217ka (https://doi.org/10.55876/gis8.240217ka) consisting of human SARS-CoV-2 sequences from South Korea and Florida, USA, collected on 15 December 2021.

Each coding region was than compared separately following gene annotation provided in the SARS-CoV-2 Wuhan-Hu-1 (Accession Number NC_045512) GenBank data. The sequences bearing long track of 'NNNN' in each gene coding region were not included in the polymorphic amino acid sequence analysis.

Evolutionary analysis of spike coding region of all Delta variant of complete and high coverage genome sequences of SARS-CoV-2 collected from dog and cat and human origin from Europe, Asia and North America collected in 2023, June 2022 and 15 June 2021. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura 1980). In this analysis, we included all human virus data from Europe, Asia, and North America if the number was under 20; otherwise, we sampled maximum 20 data from each area in the specific time frame. only five samples collected on 15 June 2021 from Europe, Asia and North America were included.

## RESULTS

Total number of complete and high coverage sequence data of Delta variant of SARS-CoV-2 collected from dog and cat as well as those collected from human in Europe, Asia, and North America in 2023, June 2022, dan 15 June 2021 available in GISAID database is presented in Table 1. Following purposive sampling of human origin sequences collected on 15 June 2021, total number of whole genome sequences was 156. The Wuhan-Hu-1 (Accession Number NC_045512) standard strain was added in the analysis. The pattern of insertion/deletion was examined and show Del22005-22010 and Del27055-27067, as well as substitution compared to Wuhan-Hu-1 strain at T19R, E157G, F158Del, F159Del, and T482K in spike gene, D63G, R203M, and D377Y in NP, G5063S and P5401L in ORF1AB, V82A and T120I in ORF7A, as well as D119Del and F120Del in ORF8 (not shown).

Unique residues of Delta variant of SARS-CoV-2 collected from dog and cat in various gene or coding region and number of dog and cat viruses harbouring respective residues are shown in Fig. 1. There were five residues in ORF1AB, namely 1240/I, 1525/V, 3056/I, 3750/I, and 5534/I, one in ORF3A, namely 113/S, and one in ORF8, namely 66/V, that occurred in dog and cat origin sequences from South Korea, while one marker of 5658/V occurred in dog and cat samples from USA. The second dataset (EPI_SET_240217ka) consisting of human SARS-CoV-2 sequences from South Korea and Florida, USA, collected in 15 November 2021 shows that the residues also occurred in human SARS-CoV-2 sequences. This result shows that there is no unique residue of Delta variant of SARS-CoV-2 collected from dog and cat.

**Table 1:** Total number of complete and high coverage sequence data of Delta variant of SARS-CoV-2 collected from dog and cat as well as those collected from human in Europe, Asia, and North America in 2023, June 2022, dan 15 June 2021 available in GISAID database

| Species | Location origin | Sampling date | Number of samples available in GISAID | Number of samples included in analysis | Data set ID |
|---|---|---|---|---|---|
| Dog | All | All | 30 | 30 | 240216nc |
| Cat | All | All | 27 | 27 | 240216nc |
| Human | Europe | 2023 | 5 | 5 | 240216nc |
| Human | Europe | June 2022 | 13 | 13 | 240216nc |
| Human | Europe | 15 June 2021 | 2991 | 20 | 240216nc |
| Human | Asia | 2023 | 8 | 8 | 240216nc |
| Human | Asia | June 2022 | 3 | 3 | 240216nc |
| Human | Asia | 15 June 2021 | 280 | 20 | 240216nc |
| Human | North America | 2023 | 2 | 2 | 240216nc |
| Human | North America | June 2022 | 8 | 8 | 240216nc |
| Human | North America | 15 June 2021 | 552 | 20 | 240216nc |
| Human | South Korea | 15 November 2021 | 75 | 75 | 240217ka |
| Human | Florida USA | 15 November 2021 | 102 | 102 | 240217ka |

| Virus strain, species origin, country origin, EPI ISL Acc. No., collection date | ORF1AB | | | | | | ORF3A | ORF8 |
|---|---|---|---|---|---|---|---|---|
| | 1240 | 1525 | 3056 | 3750 | 5534 | 5658 | 113 | 66 |
| dog10/South Korea/S-081/2021\|EPI ISL 15727702\|2021-08-20 | I | V | I | I | I | | S | V |
| dog11/South Korea/07-G-N-01/2021\|EPI ISL 15775591\|2021-08-10 | I | V | I | I | I | | S | |
| dog12/South Korea/S-079/2021\|EPI ISL 15775595\|2021-08-10 | I | V | I | I | I | | S | |
| dog6/South Korea/08-G-01/2021\|EPI ISL 15727697\|2021-12-10 | I | V | | | | | S | |
| dog7/South Korea/S-054/2021\|EPI ISL 15727699\|2021-07-14 | I | V | I | I | I | | S | V |
| dog8/South Korea/S-059/2021\|EPI ISL 15727700\|2021-07-20 | | V | I | I | I | | S | V |
| cat10/South Korea/S-050/2021\|EPI ISL 15775584\|2021-07-11 | I | V | I | I | I | | S | |
| cat11/South Korea/S-076/2021\|EPI ISL 15775585\|2021-08-08 | I | V | I | I | I | | S | |
| cat12/South Korea/S-093/2021\|EPI ISL 15775586\|2021-09-19 | I | V | I | I | I | | S | |
| cat13/South Korea/S-098/2021\|EPI ISL 15775588\|2021-10-08 | | V | I | I | I | | S | |
| cat30/South Korea/S-097/2021\|EPI ISL 15775587\|2021-10-08 | | V | I | I | I | | S | |
| cat6/South Korea/S-049/2021\|EPI ISL 15727691\|2021-07-02 | I | V | I | I | I | | S | V |
| cat7/South Korea/S-061/2021\|EPI ISL 15727692\|2021-07-22 | I | V | I | I | I | | S | V |
| cat8/South Korea/S-077/2021\|EPI ISL 15727694\|2021-08-08 | I | V | I | I | I | | S | V |
| cat9/South Korea/S-082/2021\|EPI ISL 15727695\|2021-08-24 | I | V | I | I | I | | S | V |
| dog16/USA/FL-21-025847-001/2021\|EPI ISL 5761515\|2021-08-25 | | | | | | V | | |
| dog17/USA/FL-21-025578-001/2021\|EPI ISL 5761517\|2021-08-18 | | | | I | | | | |
| dog18/USA/FL-21-022193-001/2021\|EPI ISL 5761526\|2021-07-26 | | | | I | | | | |
| dog2/USA/AZ-TG1054469/2021\|EPI ISL 12543431\|2021-12-03 | | | | | | V | | |
| dog20/USA/NJ-21-027164-001/2021\|EPI ISL 5781752\|2021-09-05 | | | | | | V | | |
| dog21/USA/GA-21-027601-001/2021\|EPI ISL 5781753\|2021-08-26 | | | | | | V | | |
| cat21/USA/CA-21-025577-001/2021\|EPI ISL 5761535\|2021-08-17 | | | | | | V | | |
| cat22/USA/WY-21-028404-001/2021\|EPI ISL 5781756\|2021-09-09 | | | | | | V | | |
| cat24/USA/ID-21-032645-001/2021\|EPI ISL 6088089\|2021-10-16 | | | | | | V | | |
| cat25/USA/CA-21-035224-002/2021\|EPI ISL 7974436\|2021-11-15 | | | | | | V | | |
| cat26/USA/IA-21-036031-001/2021\|EPI ISL 7974437\|2021-11-10 | | | | | | V | | |

**Fig. 1:** Virus strains harbouring probable molecular marker of adaptation of SARS-CoV-2 in dog and cat with the country of origin and collection date.

Evolutionary analysis of spike coding region of all Delta variant of complete and high coverage genome sequences of SARS-CoV-2 collected from dog and cat and human origin from Europe, Asia, and North America collected in 2023, June 2022 and 15 June 2021 is shown in Fig. 2 Dog and cat origin viruses clustered in Loop 1, 2, and 3, as well as spread along the tree. Loop 1 consists of cat6, 7, 12, 13, and 30 as well as dog 7, 12, 17, and 18. The cat21, 22, 24, 25, and 26 as well as dog2, 20, and 21 located in the Loop 2. The other cluster of cat16, 19, 23, 27, and 28 with dog14 and 22 formed Loop 3.

## DISCUSSION

Due to a huge number of sequencing data, we only analysed human SARS-CoV-2 collected in 2023, June 2022, dan 15 June 2021 in Europe, Asia, and North America in 2023, June 2022, dan 15 June 2021. The samples were then purposively selected of the fist and the last 10 from the database. While all available dog and cat data were included, the number of samples is large enough to draw a conclusion. In confirming the unique markers discovered in dog and cat in South Korea and USA, we even analysed all human SARS-CoV-2 sequences from South Korea and Florida, USA, collected in 15 November 2021.

The main finding is evidence of spill-over of SARS-CoV-2 from human to dog and cat. No species-specific markers, that indicates a probable species adaptation marker of the pandemic Covid-19 virus in dog and cat, could be identified. The markers found in many dog and cat samples (Fig. 1) is confirmed to be area specific markers. Five unique residues in ORF1AB, namely 1240/I, 1525/V, 3056/I, 3750/I, and 5534/I, one in ORF3A, namely 113/S, and one in ORF8, namely 66/V, that occurred in dog and cat origin sequences from South Korea also occurred in human SARS-CoV-2 located in South Korea at the same space of time. The order marker of 5658/V occurred in dog and cat samples from USA also happened in human samples in USA during the same time period.

Surveillance for genetic markers of adaptation could help to predict the risk of disease emergence (Pepin et al. 2010). In influenza virus, species adaptation markers or signature substitutions have resulted from virus adaptation to the new host (de Wit et al. 2010; Lloren et al. 2017). The absent of species adaptation marker in dog and cat is reasonable. These animals are solitary except in wild where the animals, especially dog, are living in close contact with each other in a pack. As companion animals, dog and cat mingle more with people than other animals, especially in the pandemic where people movement is limited. Moreover, from the GISAID metadata we understand that the animal samples were taken individually and none from population. Since dog and cat experienced a mild or sub-clinical course following SARS-CoV-2 infection, the need to test these companion animals is not of high priority. More samples with complete genome data should bring evidence of signature markers for dog and cat adaptation of SARS-CoV-2.

Previous observation is valid that Delta variant has unique deletions and substitutions in many gene coding region. We observed almost all data of Delta variant dataset in this study has deletion at Del22005-22010 and Del27055-27067, as well as substitution compared to Wuhan-Hu-1 strain at T19R, E157G, F158Del, F159Del, and T482K in spike gene, D63G, R203M and D377Y in NP, G5063S and P5401L in ORF1AB, V82A and T120I in ORF7A, as well as D119Del and F120Del in ORF8 as earlier described as unique Delta variant markers (Suardana et al. 2023; Suharsono et al. 2023).

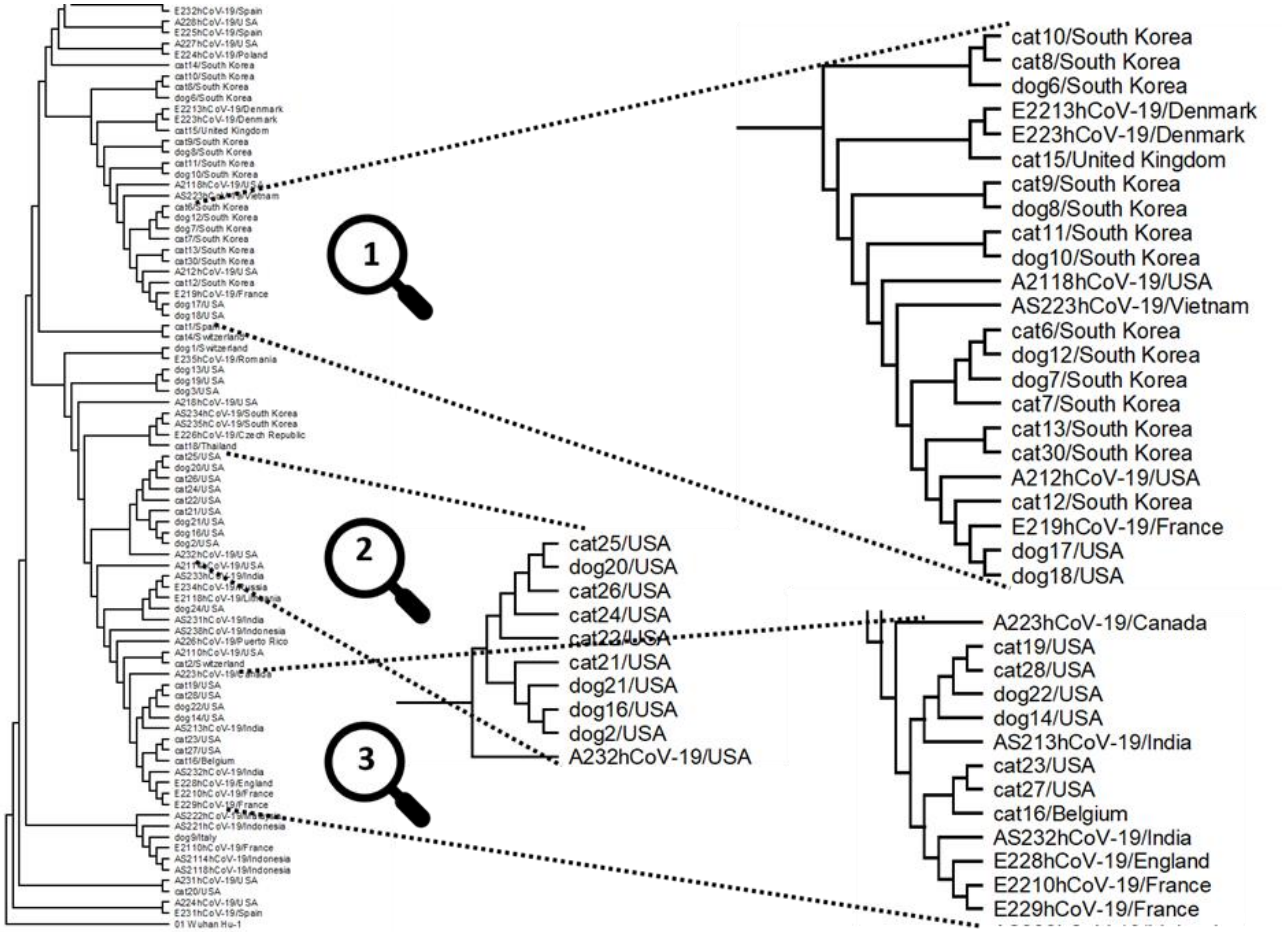


**Fig. 2:** Evolutionary analysis of spike coding region of all Delta variant of complete and high coverage genome sequences of SARS-CoV-2 collected from dog and cat and human origin from Europe, Asia, and North America collected in 2023, June 2022, and 15 June 2021. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-7268.15) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. The tree was rooted to SARS-CoV-2 Wuhan-Hu-1 strain. Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021). Three major clusters of sequences collected from dog and cat are looped as Loop 1, Loop 2, and Loop 3. The taxon names were numbered to generate unique ID for Clustal Omega analysis and easy tracing in data interpretation. The taxon names include the location (E for Europe, AS for Asia, and A for north America), the first two number means collection year (21 for 2021, 22 for 2022, and 23 for 2023).

The phylogeny was conducted to bring a rapid overview of possible relationship between taxon. The phylogeny in this was not a quantitative analysis to pin point the origin of certain taxon. The gene fragment could be not appropriate to solicit such ancestor-taxon relationship in Covid-19. As described before, inferring a reliable phylogeny due to the large number of sequences in conjunction with the low number of mutations is complicated (Morel et al. 2020). Moreover, applying spike coding region might not suitable for SARS-CoV-2 phylogenetic analysis. The N gene could fit better for that purpose as the global analysis has reported that the N gene has the highest mutation rate in SARS-CoV-2 structural protein coding region (Abavisani et al. 2022). Nevertheless, applying the phylogeny based on spike sequence showed that there is only partial concordance of unique residues in dog and cat with its human counterpart (Fig. 1 and 2). The dog6- 8, 10-11, 12 and cat 6-9, 10-13, 30, that Harbor South Korean markers, are located in separated loop in Fig. 2. The phylogeny also shows the global travel of SARS-CoV-2. Distancing virus form a cluster which reflect their close genetic distance. As it has been reported previously, the constructed phylogenetic tree indicates possible multiple independent introduction events (Goletic et al. 2021; Tabibzadeh et al. 2020; Ul-Rahman et al. 2023) due to global travel. This again demonstrate although travel restrictions have indeed led to important changes in the dynamics of the early phases of the COVID-19 pandemic (Grepin et al. 2021), national borders remain permeable.

Being solitary animals and the low priority to test these companion animals and conducting full genome sequencing hamper us to detect any species adaptation signal of the SARS-CoV-2 in dog and cat. Lack of history of clinical course of the dog and cat in GISAID metadata leads to the difficulty soliciting the possible adaptation process on the pandemic virus.

In conclusion, species-specific marker, that indicates a probable species adaptation marker of the pandemic SARS-CoV-2 in dog and cat, could not be identified. The markers found in many dog and cat samples is confirmed to be location specific markers that occurred in human

origin SARS-CoV-2 sequence data.

**Data availability**

The first data set ID is EPI_SET_240216nc (https://doi.org/10.55876/gis8.240216nc). The second dataset EPI_SET_240217ka (https://doi.org/10.55876/gis8.240217ka) consisting of human SARS-CoV-2 sequences from South Korea and Florida, USA, collected in 15 November 2021 as well as human in Europe, Asia, and North America in 2023, June 2022, dan 15 June 2021. All genome sequences and associated metadata in this dataset are published in GISAID's EpiCov database. To view the contributors and each individual sequence with details such as accession number, virus name, collection date, originating lab and submitting lab and the list of authors, visit https://doi.org/10.55876/gis8.240216nc and https://doi.org/10.55876/gis8.240217ka.

**Conflict of interest**

The authors have declared no conflicts of interest.

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**Author contributions**

Ida B. K. Suardana and Gusti N. Mahardika contributed to the conception. Ida B. K. Suardana, Bayu K. Mahardika, contributed to the acquisition, analysis, and interpretation of data; Ida B. K. Suardana and Gusti N. Mahardika drafted the manuscript. All authors reviewed the manuscript and approved the version to be published.

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