Comparison of SARS-CoV-2 VOC 202012/01 (UK variant) and D614G variant transmission by different routes in Syrian hamsters

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Abstract

Many SARS-CoV-2 variants of concern has been reported recently which were linked to increased transmission. In our earlier study on virus shedding using VOC 202012/01(UK variant) and D614G variant in hamster model, we observed significantly higher viral RNA shedding through nasal wash in case of UK variant. Here, we have compared the transmission of both the UK and D614G variant by various routes in Syrian hamsters to understand the transmission efficiency of the variant. The study demonstrated comparable transmission efficiency of both UK and D614G variants of SARS-CoV-2 in Syrian hamsters.

Short Communication

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Running title: *Comparison of transmission of SARS-CoV-2 variants in Syrian hamsters*

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Summary

Many SARS-CoV-2 variants of concern has been reported recently which were linked to increased transmission. In our earlier study on virus shedding using VOC 202012/01(UK variant) and D614G variant in hamster model, we observed significantly higher viral RNA shedding through nasal wash in case of UK variant. Here, we have compared the transmission of both the UK and D614G variant by various routes in Syrian hamsters to understand the transmission efficiency of the variant. The study demonstrated comparable transmission efficiency of both UK and D614G variants of SARS-CoV-2 in Syrian hamsters.

Keywords

SARS-CoV-2, UK variant, VOC202012/01, D614G, transmission, hamster

Main text

SARS-CoV-2 virus has accumulated numerous genetic changes on circulation all over the world. Some of these mutations have the potential to change the virus characteristics like infectiousness, transmissibility, severity of disease and can have impact on diagnostics, vaccines and therapeutics (World Health Organisation, 2021). SARSCoV-2 variant with a D614G substitution which emerged in the first quarter of the year 2020 supplanted the initial SARS-CoV-2 strain showing its increased fitness to become the dominant strain circulating globally. Recently, many variants of concern (VOC) were reported from United Kingdom, South Africa, Brazil etc. which were linked to increased transmission, disease severity and vaccine escape mutants (Davies et., 2020). To categorize a variant as VOC, various risk elements like increased transmissibility, morbidity, mortality, immunity escape factors needs to be studied. Scanty information is available on these aspects about the variants.

In our earlier study on virus shedding using VOC 202012/01(UK variant) and D614G variant in Syrian hamster model, we observed significantly higher viral RNA shedding through nasal wash in case of UK variant (Mohandas et., 2021). Direct contact, aerosol and fomite routes of transmission of SARS-CoV-2 has been established in hamster model (Sia et al., 2020). Here we have compared the transmission of both the UK and D614G variant by various routes in Syrian hamster model to understand whether the high viral RNA shedding through nasal cavity in hamsters infected with UK variant could enhance the transmission efficiency of the variant.

The study was approved by the Institutional Animal Ethics (No. NIV/IAEC/2021 /MCL/01) and Biosafety Committee (No. NIVIBSC/05.01.2021/02) of Indian Council of Medical Research (ICMR) -National Institute of Virology (NIV), Pune and all the experiments were performed as per the institute ethical guidelines (CPCSCEA guidelines, Government of India). The study was performed in the containment facility with a total of 36 male hamsters of 6-8 week age housed in individually ventilated cages. Nine hamsters each were intranasally infected with two SARS-CoV-2 variants i.e., UK variant (hCoV-19/India/NIVP1 20203522/2020, GISAID identifier: EPL_ISL_825088)) and a SARS CoV-2 variant isolated during March 2020 from Italian tourists which possess D614G mutation (hCoV-19/India/2020770/2020, GISAID identifier: EPL_ISL_420546)) with 0.1 ml of virus of 105.5 TCID50/ml under isoflurane anaesthesia and were used as donor hamsters for studying transmission via direct contact, aerosol and fomite routes (Sarkale et al., 2020; Yadav et al., 2021). All the experiments were performed in triplicates for both the variants. SARS-CoV-2 genomic RNA (gRNA) load were tested in the nasal wash, throat and faecal swab samples collected from the contact hamsters on every alternate day till 14 days post exposure (DPE) using E gene quantitative real-time RT-PCR as described earlier (Choudhary et al, 2020). Virus titration was also performed for the nasal wash samples in Vero CCL81 cells. The donor hamsters infected with UK variant showed progressive weight loss with the maximum average weight loss of 11 $\pm$ 2.82 % [mean $\pm$ standard deviation(S.D)] on day 6 whereas the donor hamsters infected with D614G variant showed a maximum average weight loss of -6.66$\pm$ 1.36 % on day 8. The donor hamsters showed regain of weight thereafter. All the donor hamsters, bedding, cage surfaces and water bottle nozzle samples from cages used for fomite transmission study were tested for gRNA to ensure the presence of the virus before exposure (Table 1).

Twenty four hour post infection, 3 donor hamsters were co-housed with a naive hamster (referred to as contact hamster further) in 1: 1 ratio in a new cage to study direct transmission and were observed till 14

DPE for body weight change and any respiratory signs. The contact hamsters exposed with UK variant showed maximum average weight loss of -2.93 ± 0.34 % and with D614G variant showed -4.8± 3.13 % on 8 DPE (Fig. 1A). The contact hamsters exposed with both variants showed viral gRNA positivity in the throat swab, nasal wash and faecal samples from 2nd DPE and peak average viral gRNA load by 2nd to 4th DPE (Figure 1B-D). This is similar to the pattern of detection reported in intranasal inoculated hamsters with both variants (Mohandas et al., 2021; Hou et al., 2020). Titration of nasal wash samples showed consistent presence of virus till 10DPE with comparable titre in hamsters exposed with both variants (Fig. 1E).

As the direct contact transmission, could be contributed by aerosol and fomites, we assessed these routes of transmission alone. To assess the aerosol transmissibility, SARS-CoV-2 infected hamster after 24 hours post infection was co-housed in a modified individual ventilated cage with partition (which allows airflow) with a naive hamster for 8 hours. The contact hamsters were housed in new cages and were observed for 14 days. One hamster from the aerosol contact group of D614G variant did not show any body weight loss and showed very low or negligible amount of viral RNA load in the samples. The other hamsters of the aerosol contact group of UK variant and D614G variant showed a peak average weight loss of -8.9% on 8 DPE and -4.2% on 10 DPE respectively (Fig. 1A). The viral gRNA detection was observed from 2ndDPE and the peak average viral gRNA detection in the nasal wash and faecal samples was observed on 6 DPE for hamsters exposed with both variants and higher viral load till 10 days (Fig. 1B-D). The TCID50/ml of the nasal wash samples from 6 to 10DPE showed higher titres (Fig.1E).

For the fomite transmission study, 3 naive hamsters were housed in different cages with soiled bedding of SARS-CoV-2 infected hamster housed for 48 hours following infection. Progressive body weight loss was also observed in fomite contact hamsters of both the variants till 14 days (Fig. 1A). The peak average viral gRNA in fomite contact hamsters varied from 6 to 8 DPE in case of UK variant and 4 to 6 DPE with D614G variant (Fig 1B-D). This is in contrary to an earlier study which reported less efficient fomite transmission by SARS-CoV-2 in hamsters (Sia et al., 2020). Even though viral gRNA could be detected till 14DPE, live virus particles could not be detected from 6DPE in case of D614G variant contacts in contrast to UK variant contacts where it could be detected till 10DPE (Fig.1E) .

Anti-SARS-CoV-2 IgG antibodies by anti-SARS CoV-2 IgG ELISA could be detected in all the donor and contact animals on 21 DPE except one contact hamster of the D614G variant contact of the aerosol group which showed negligible viral gRNA load (Mohandas et al., 2020). The day wise comparison of the body weight loss and the viral shedding pattern in contact hamsters by both variants on Mann-Whitney test did not show any statistical significance. Also the comparison of different routes of transmission by each variant on Kruskal Wallis test also did not show any statistical significance.

The virus detection in contact hamsters were seen as early as on 2nd DPE for both variants indicating faster transmission and a maximum infectious period of 10 days by all routes. The peak viral gRNA levels were comparable among different transmission routes whereas the peaks of detection by the aerosol and fomite route were found extended and with more variations among contact animals. The percent body weight loss also varied among different routes of transmission. This could be due to difference in the amount of virus dose exposure by these routes. Earlier research have shown that lower and higher dose of virus inoculums show comparable viral gRNA loads in hamsters but the lung lesions and body weight loss vary with virus dose (Mohandas et al., 2020; Ryan et al., 2021)

In conclusion, the transmission of SARS-CoV-2 variants could be established in Syrian hamsters by direct, aerosol and fomite routes as evident by the body weight loss, detection of viral gRNA/live virus in the samples and anti-SARS-CoV-2 IgG antibodies in the contact hamsters. The study demonstrated comparable transmission efficiency of both UK variant and D614G variants of SARS-CoV-2 in Syrian hamsters.

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Conflict of interests

The authors have declared no conflicts of interest.

Author's contributions

PDY and SM conceived, designed and performed the animal experiments. PDY, AS, KL and SH performed the laboratory investigations and standardizations. DN performed the statistical analysis. PS performed the titration experiments. SM wrote the draft manuscript. PDY, SK, KL reviewed and substantively revised the manuscript. All the authors have read the manuscript and agree to its contents.

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Table 1: SARS-CoV-2 viral gRNA load of donor hamsters and the cages used in the study at 24 hours post virus infection.

Study	**Sample details**	**Sample**	**Log_{10} viral gRNA copies/ml**	**Log**
			VOC 202012/01	**D6**
Direct transmission	Donor hamster 1	Throat swab	8.40	7.83

Study	Sample details	Sample	Log_{10} viral gRNA copies/ml	Log
		Nasal wash	9.85	9.29
		Faeces	8.33	7.10
	Donor hamster 2	Throat swab	7.66	7.90
		Nasal wash	10.24	9.40
		Faeces	7.51	4.99
	Donor hamster 3	Throat swab	8.01	7.39
		Nasal wash	9.64	9.11
		Faeces	5.10	6.22
Aerosol transmission	Donor hamster 1	Throat swab	7.31	8.41
		Nasal wash	9.68	10.0
		Faeces	5.31	7.06
	Donor hamster 2	Throat swab	8.71	7.65
		Nasal wash	10.40	9.86
		Faeces	7.73	6.79
	Donor hamster 3	Throat swab	7.09	7.95
		Nasal wash	9.84	9.38
		Faeces	6.96	6.72
Fomite transmission	Donor cage 1	Bedding	6.67	6.05
		Cage surfaces & water bottle nozzle	7.24	6.91
	Donor cage 2	Bedding	6.36	5.00
		Cage surfaces & water bottle nozzle	8.33	6.59
	Donor cage 3	Bedding	6.19	5.59
		Cage surfaces & water bottle nozzle	7.13	6.73

Figure legend

Figure 1: Percent body weight change and SARS-CoV-2 viral load in the hamsters post exposure. (A) The body weight change in hamsters following exposure with SARS-CoV-2 infected hamsters by direct, aerosol and fomite contact. Viral gRNA load in (B) throat swab (C) nasal wash (D) faeces in hamsters exposed by direct, aerosol and fomite contact. (E)Viral load in nasal wash samples of contact hamsters exposed by direct, aerosol and fomite contact estimated by titration in Vero CCL-81 cells expressed in TCID50.