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Persistence and viability of SARS-CoV-2 lineages B.1 and EG.5.1 on plant-based foods: Implications for food safety and transmission

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ABSTRACT

Five years post-emergence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) the route of transmission through fomites is still discussed. Therefore, in the present study ten plant-based foods (apple, grape, fennel, carrot, lettuce, celery, tomato, radish, rocket, and cucumber). The matrices were artificially contaminated by spraying 50 μL of viral solution, and RNA persistence and viral viability were assessed via RT-qPCR, Vero E6 cell inoculum, and TCID50 assays, and analysed at seven time points (0.5–96 h post-contamination). The EG.5.1 variant demonstrated longer environmental stability, with viral RNA detectable on all matrices up to 96 h post-contamination except for celery (24 h), while lineage B.1 RNA was less persistent, particularly on carrot and rocket (24–48 h, respectively). Conversely, B.1 remained viable up to 4 h on most matrices, while EG.5.1 viability ceased by 30 min on grape, fennel, lettuce, and celery.

These results suggest that while food preparation practices such as washing and thermal processing effectively reduce the risk of viral presence, post-processing contamination may facilitate fomite-mediated transmission, particularly via raw products. These findings contribute to the ongoing evaluation of SARS-CoV-2 transmission routes and highlight the importance of stringent hygiene practices throughout the food supply chain.

1. Introduction

1.1. SARS-CoV-2 transmission

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the aetiological agent responsible for Coronavirus Disease 2019 (COVID-19), which emerged in late December 2019 in China and rapidly spread worldwide, becoming a pandemic. Since May 2023, the World Health Organization (WHO) has declared that COVID-19 no longer represents an international public health emergency but requires attention, especially aiming at long-term management, with a strategic response plan 2023–2025 (WHO, 2023a).

Since the beginning of the pandemic, the study of SARS-CoV-2 transmission has been one of the most addressed topics (Arienzo et al., 2023; Meyerowitz et al., 2021). It was demonstrated that the primary route of infection is through direct transmission via respiratory droplets (Cevik et al., 2020), however, indirect transmission via fomites has been the subject of several studies. Although recent evidence suggests fomite transmission is less significant than respiratory droplets (Meyerowitz et al., 2021; Li et al., 2023, (CDC, 2021), the risk of fomite-mediated transmission is still unclear, and the extent of the risk associated with this route remains inconclusive. Therefore, understanding the persistence time of the virus on different surfaces of daily use and the possibility of infection via the surface remains of utmost importance (Chin et al., 2020; Fusco et al., 2023; Kwon et al., 2021; van Doremalen et al., 2020; Xie et al., 2020). COVID-19 is mainly characterised by respiratory illness, albeit several studies have also reported gastrointestinal (GI) symptoms (Chen et al., 2022; Giobbe et al., 2021). Indeed, SARS-CoV-2

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is able to attack host cells through the interaction between the virus Spike glycoprotein (S) and the host angiotensin-converting enzyme 2 (ACE2) receptor, which is highly expressed in lung, heart, and gastrointestinal cells (Shirbhate et al., 2021; Xiao et al., 2020), thus justifying the involvement of the GI tract and the infectivity of faecal SARS-CoV-2 (Termansen & Frische, 2023). In particular, it has been evidenced that ACE2 receptor is abundantly expressed in the glandular cells of gastric, duodenal, and rectal epithelia, supporting the entry of SARS-CoV-2 into the host cells and, following viral entry, supported by host proteases such as TMPRSS2 or cathepsins, active replication within gastrointestinal epithelial cells has been demonstrated, suggesting a potential route for fecal-oral transmission (Minami et al., 2023; Xiao et al., 2020). Indeed, as soon as the virus is absorbed orally, it replicates in the glands and oral mucosa (Hoffmann, 2023). During this process, saliva protects the virus, allowing SARS-CoV-2 to enter the gut, remain alive, and replicate in intestinal epithelial cells (Minami et al., 2023). Altogether, these findings have raised the concern of oral infection and faecal-oral transmission, as well as the hypothesis that contaminated foods and drinks may play a role in SARS-CoV-2 transmission (Chen et al., 2022; (Esseili et al., 2022); Saulnier et al., 2023; Termansen & Frische, 2023).

1.2. Food as fomites

Food and food packaging may behave as fomites, allowing the virus to be transmitted between food workers and consumers (Günther et al., 2020; Xie et al., 2020). Indeed, food can be exposed to pathogens during food production and preparation, such as washing with contaminated water or direct contamination by food operators (Ceylan et al., 2020; Le Guyader et al., 2008), as also demonstrated for other viruses, such as noroviruses, hepatitis A virus and rotaviruses, which can contaminate food and remain infectious even for 4 weeks (Stals et al., 2012). Regarding SARS-CoV-2, at the beginning of the pandemic, up to 55 % of COVID-19 cases in China were associated with Wuhan markets (Bai et al., 2021), while in the United States, many cases diagnosed between April and May 2020, came from food production facilities (Dyal et al., 2020). Furthermore, some studies have shown the presence of SARS-CoV-2 RNA on the surfaces of different food and their packaging, which may indicate a possible transmission through imported frozen food products (Liu et al., 2020; Pang et al., 2020). During their preparation, foods come into contact with several surfaces that may be contaminated with pathogens (Pressman et al., 2020; Warnes et al., 2015).

1.3. Study rationale

The contact between infected surfaces and foods, as well as operators handling them, may raise the risk of infection by self-inoculation via the mouth, nose, or eyes (De Rose et al., 2020; FSA, 2020; USDA, 2020). Although the European Food Safety Authority (EFSA) in 2020 highlighted the absence of evidence of SARS-CoV-2 transmission through food consumption (EFSA, 2020), some studies have linked this event to food production and storage (Godoy et al., 2021; Han et al., 2021). Indeed, viral RNA has been detected in frozen food items such as fish, meat and seafood (Economou et al., 2021; Nakat & Bou-Mitri, 2021; Yekta et al., 2021; Zhan, 2020). However, literature data suggest that the ability of SARS-CoV-2 to survive in food matrices is still largely unknown. Fukuta et al. (2021) evaluated the viability of the virus in common beverages and body, and it was observed that the infectious virus could be recovered up to 77 days in liquids such as milk and water, while in other samples the persistence of viral RNA up to 28 days was demonstrated (Fukuta et al., 2021). Similarly, a study conducted on artificially contaminated berries stored under different conditions of SARS-CoV-2 showed that the virus remains infectious on frozen berries for at least 28 days without a significant reduction in infectivity (Essei Y. Li et al., 2022). These data highlight how SARS-CoV-2 can maintain remarkable stability under certain conditions, underscoring the importance of continuing to implement appropriate control measures and further research into aspects of indirect transmission in the context of food sales, production, and consumption, or in the cold chain industry. Therefore, the present study aimed to analyze and compare the persistence and viability of SARS-CoV-2 lineages B.1 and EG.5.1 on ten not heat-treated different plant-based food such as fruit and vegetable matrices.

2. Materials and methods

2.1. Artificial contamination

In the present study, 10 plant-based food matrices, apple, grape, fennel, carrot, lettuce, celery, tomato, radish, rocket, and cucumber, were artificially contaminated according to a protocol already described by the same authors among surfaces (Fusco et al., 2023), with small changes. Fig. 1 reports a flowchart of the study.

Briefly, fresh, organic produce purchased from a retail supermarket was washed and cut into 4–5 cm sections using sterile scalpels into seven equal portions of approximately 4–5 cm, each corresponding to a specific post-contamination sampling time, identified as 0.5 h (30 min), 4 h, 8 h, 24 h, 48 h, 72 h, and 96 h. The sections were first tested for the absence of SARS-CoV-2 by RT-qPCR as already described (Cardillo et al., 2021) and reported below. Sections were exposed to 253.7 nm UV-C light for 15 min at 31.2 mW/cm^2 (Sankyo Denki co., ltd., Kanagawa, Japan).

Next, each section was placed in a different Petri dish, corresponding to the relative post-contamination (p.c.) sampling time.

The artificial contamination was carried out using SARS-CoV-2 lineage B.1 (hCoV-19/Italy/CAM-INMI-32803-66/2020) and EG.5.1 (hCoV-19/Italy/CAM-C-27-11-23-S7/2023) variant, which were previously titrated using Endpoint Dilution Assay (TCID50/mL) according to the Spearman-Kärber method (Kärber, 1931) and quantified by RT-qPCR. Next, 50 μL-viral solutions were obtained containing a viral titre of 10^5 TCID50/mL SARS-CoV-2, corresponding to 4.68E+09 copies/mL for SARS-CoV-2 lineage B.1, and 6.90E+09 copies/mL for EG.5.1 variant, respectively. Therefore, viral aliquots of 50 μL were sprayed onto each section through a commercially available atomizer (Farmalabor, Italy) to mimic the nebulization of sneeze/cough aerosol. The samples were then incubated at 22.5 °C and with relative humidity (RH) of 58.6 % to mimic median indoor conditions. In addition, separate petri dishes with sections contaminated with 50 μL of virus-free culture medium were set up for all tested matrices to normalize data with negative controls.

Next, sterile flocked swabs were used for sampling at the seven specific p.c. times, by rubbing the food surface for 10 s with horizontal, vertical, and transverse movements, for a total of 30 s, and placed in Universal Transport Medium (UTM) (Copan, Brescia, Italy), as per the indications of the Food Standard Authority (FDA, 2022).

2.2. Virological examinations

Certified Vero E6 cells (ATCC Vero C1008) (D'Argenio et al., 2019) were previously cultured using EMEM culture medium (Gibco, Life-Technologies, Europe B.V. Bleiswijk, Netherlands) integrated with 10 % foetal bovine serum (FBS) (Gibco, Life Technologies, Europe B.V. Bleiswijk, Netherlands), 1 % antibiotic-antimycotic (Gibco, Life-Technologies, Europe B.V. Bleiswijk, Netherlands) and 2 mM L-glutamine (Gibco, Life Technologies, EuropeB.V. Bleiswijk, Netherlands) according to the European Collection of Authenticated Cell Cultures (ECACC) protocol until they reached 80 % confluence and were used to evaluate the viability of the two viruses at each sampling time. All the experiments were performed in triplicate and normalised with positive and negative controls, represented by free virus-EMEM inoculum. Next, 24-well plates were prepared by seeding approximatively 150,000 cells/well until 80 % cell confluence was reached. Next, 1 mL of UTM

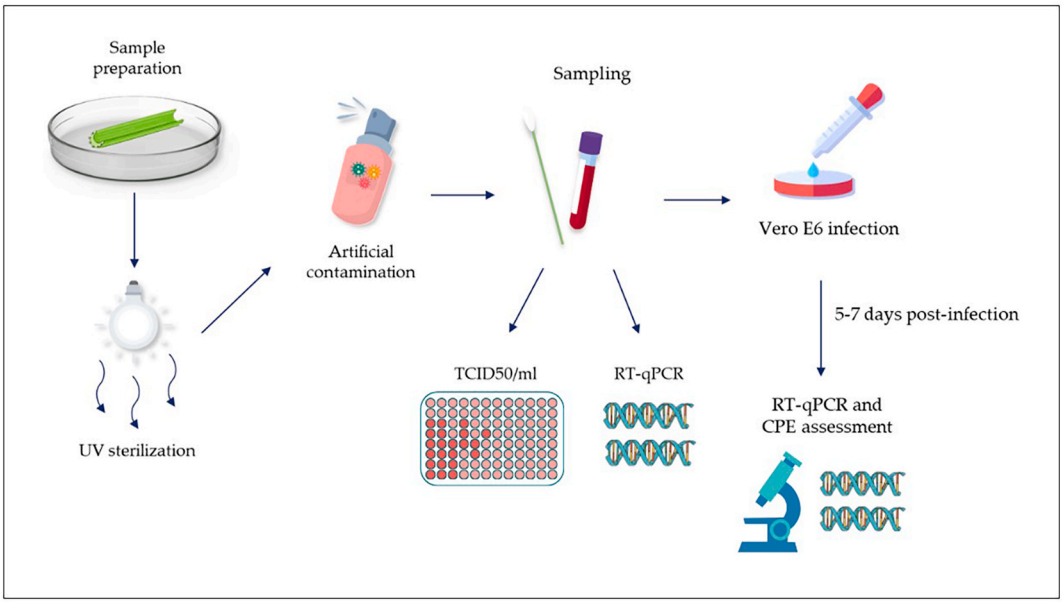


Fig. 1. Experimental workflow for SARS-CoV-2 contamination and analysis on plant-based foods.
Samples were dissected and sterilised using UV light; SARS-CoV-2 lineage B.1 and EG.5.1 were used to perform the artificial contamination of the 10 different food matrices. Directly on the matrices, after infection, pH was evaluated (1) and at each sampling time. From 0.5 h to 96 h post-contamination, swabs were rubbed from the matrices and deposited in 1 mL of UTM medium. Next, 50 μL of UTM were collected to perform viral titration by TCID50/mL (2), 200 μL for nucleic acids extraction and subsequent RT-qPCR analysis (3), and 100 μL were used as inoculum for Vero E6 cells (4). The cell cultures were daily inspected up to 7 days p.c. using an inverted microscope. The culture supernatant was collected after 5–7 days post-inoculum and analysed by RT-qPCR (5) and finally, when at least 50 % of cytopathic effect (CPE) occurred, it was assessed with microscopy analyses (6).

was filtered through 0.45 μm pore size syringe filter (Millipore, Thermo Fisher Scientific, Waltham, MA, USA) and 100 μL were used as cell inoculum, incubated for 30 min at 37 °C with 5 % CO2 and, finally, 1 mL complete EMEM with 2 % FBS, was added into each well. All the experiments were conducted in the presence of a negative control, using EMEM medium as inoculum, and a positive control. Next, inverted microscope at 5X magnification (Axiovert 25 inverted microscope, Carl Zeiss, Oberkochen, Germany) using AxioVision 4.8 software, was used to inspect the monolayers after 24 h for the visualization of the cytotoxic effect and up to 7 days for the development of the cytopathic effect (CPE), defined as a set of morphological and metabolic variations that include fragmentation, cell rounding, cell fusion and loss of cell-cell adhesion (Céspedes-Tenorio & Arias-Arias, 2023).When at least 50 % CPE occurred or, after 7 days post cell infection, the supernatant was collected after three cycles of frosting and towing, and viral loads were evaluated. Finally, quantification of the infectious particles for the residual infectivity of the virus on the food matrices was carried out using TCID50 assay on Vero E6 cells. In brief, 50 μL of UTM were collected from the swab and aliquots of 10 μL were used in duplicates to infect 80 % confluent Vero E6 cells on 96-well plates with ten-fold serially diluted virus up to reach 10^{-6} final dilution. Finally, in each plate, eight wells were used as no-virus control. The infected cells were incubated at 37 °C with 5 % CO2 and daily inspected from 2 up 7 days post infection to evaluate the development of CPE by cristal violet staining (Fusco et al., 2023).

2.3. Molecular analyses

Aliquots of 200 μL were collected from UTM, cell culture and subculture cryolysates with the addition of 200 μL TRIzol (Ambion, Life Technologies, Carlsbad, CA, US) to perform nucleic acids extraction and purification with KingFisher Flex (Thermo Fisher Scientific, Waltham, MA, USA), using the MVP_2Wash_200_Flex program, following manufacturer's instructions. The elution was carried out in a 50-μL final volume and stored at −80 °C until use. RT-qPCR analysis was conducted using TaqPath COVID-19 CE-IVD RT-PCR kit (Thermo Fisher Scientific) approved by the World Health Organization (WHO, 2020), that amplifies three viral targets, ORF1ab gene (FAM), N protein (VIC), S protein (ABY) and MS2 phage (JUN) as internal control. All the reactions were performed on QuantStudio 7 Pro (Applied Biosystems, Foster City, USA) platform with same amplification settings and thermal profile reported by Cardillo et al. (2021). Viral loads were assessed by constructing a standard curve, by performing appropriate 10-fold serial dilutions in triplicate of the titrated positive control provided by the RT-PCR Kit. Next, linear regression analysis of the threshold cycle (C_t) value (y-axis) *versus* the log of the initial copy number present in each sample dilution (x-axis) was evaluated. Therefore, PCR efficiency (E) was calculated as $E = 10^{(1/slope)^{-1}}$.

Therefore, the variation in viral load from swabs to cell cultures as well as the presence of cytopathic effect (CPE), and the analysis of the viral titre by TCID50/mL, allowed us to estimate SARS-CoV-2 B.1 and EG.5.1 survival times among all the tested matrices. Furthermore, at each sampling time, food pH was evaluated with the FiveEasy Plus pH meter FP20-Std-Kit (Mettler-Toledo, Columbus, OH, US), calibrated with buffer solution at pH 4.0, pH 7.0 and pH 10.0, according to the manufacturer's instructions.

2.4. Statistical analyses

All RT-qPCR (copies/mL) experiments were performed in triplicate to ensure reproducibility of results. Raw data were transformed to logarithmic scale ($\log_{10}$), and for each experimental point, the standard error of the mean (SEM) was calculated to assess the variability of the data. Graphs were performed using GraphPad Prism version 8.0.2 software.

3. Results

3.1. RT-qPCR analysis on UTM swab

After the artificial contamination of the 10 selected plant-based food matrices with SARS-CoV-2 lineage B.1 and SARS-CoV-2 EG5.1 variant, UTM swab was analysed by RT-qPCR to assess the presence of the nucleic acids on each tested food at the seven different sampling times (0.5 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h). Results showed a different persistence of the two strains among the plant-based food matrices, also probably due to the porosity and evaluation of the foodstuff tested. Therefore, while the B.1 strain RNA was detectable up to 96 h post-contamination in six plant-based foods (apple, grape, fennel, lettuce, celery, and tomato), viral RNA was detected up to 72 h on radish and cucumber, and in carrots and rockets up to 24 and 48 h, respectively (Fig. 2A and B). Conversely, EG5.1 RNA was detectable in all the tested plant-based food matrices up to 96 h post contamination except for celery (Fig. 2C and D) (all quantitative data are reported in Table 1).

3.2. RT-qPCR on Vero E6 cell supernatant at 5–7 days post-infection

At each sampling time, a swab was administered onto the plant-based food matrices, placed into UTM and 100 μL were used to perform Vero E6 monolayer infections. When CPE developed or, in the absence of any CPE, five to seven days post-infection, the supernatant was collected from each culture deriving from a specific time, to evaluate the presence of viral RNA, as well as any change in the viral loads between the swab and the cell passage. In cell passages, SARS-CoV-2 lineage B.1 RNA was detectable up to 96 h post-infection only for apple, at 72 h p.i. for lettuce, 24 h for grape, celery, and radish, while 8 h for the other five food matrices (Fig. 3A–L). Furthermore, in the transition between swab and cell culture, it was observed an increase in the viral loads in the supernatant of UTM-infected cells within 30 min p.i. in tomato and cucumber, while within 4 h in the other eight foods. Overall, SARS-CoV-2 lineage B.1 RNA increased by 0.58–2.75 log10 copies/mL in cell supernatant compared to swab samples. Different results were obtained with the lineage EG5.1. Viral RNA was detectable at 96 h p.i. on lettuce, at 48 h on apple, grape, and tomato, at 24 h on radish and at 8 h p.i. on fennel, carrot, celery and rocket (Fig. 4A–L). Finally, a variation in viral load was observed at 30 min p.i. on grape, fennel, lettuce, and celery, at 4 h p.i. on apple, tomato, radish, rocket, and cucumber, while no change was revealed on carrot (Table 1). Overall, SARS-CoV-2 lineage EG5.1 RNA increased by 0.26–1.89 log10 copies/mL in cell supernatant compared to swab samples.

3.3. Viral titration analysis (TCID50/mL) on swab samples, CPE on VeroE6 cells and pH evaluation

In order to evaluate the viability of the virus among the different plant-based food matrices, viral titration (TCID50/mL) was carried out on UTM derived from each swab collected at different timepoints (from 0.5 to 96 h p.c.). Data obtained from SARS-CoV-2 lineage B.1 were consistent with the variation in viral loads from swabs to cultures, as well as the observation of CPE, which was detectable for all infections at 4 h except for cucumber and tomato, which was detected up to 30 min. In contrast, TCID50/mL analysis of SARS-CoV-2 variant EG.5.1

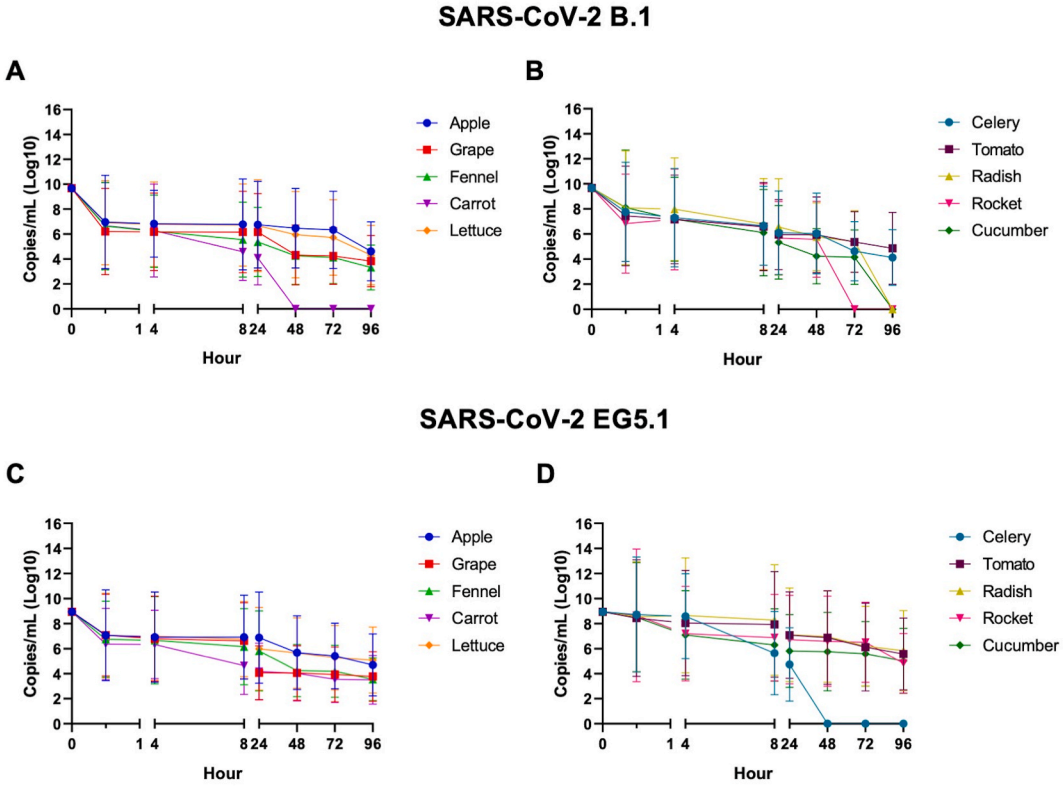


Fig. 2. Graphical representation of results obtained by RT-qPCR
For the 10 foods contaminated with SARS-CoV-2 lineage B.1 (A–B) and with EG5.1 variant (C–D). In the figure, the 10 foods are divided into two groups of five. The same five foods are present in A, C and B, D panel. The 10 foods were contaminated with the same starting amount of virus based on the strain used (A, B) and (C, D) represented by T0 in the two graphs. the evaluation was performed on UTM swabs collected at seven different times and the presence of viral RNA was evaluated. The experiment was carried out in triplicate, so the average of the three detections was then expressed in Log10. Error bars represent Standard Error Mean (SEM) from triplicate experiments. Accordingly, the RNA levels detected in the 10 foods ranged from 3.33 $\log_{10}$ copies/mL in fennel at 96 h (A) to 8.12 $\log_{10}$ copies/mL in cucumber at 30 min (B) for variant B.1, and from 3.51 $\log_{10}$ copies/mL in the carrot at 96 h (C) to 8.72 $\log_{10}$ copies/mL in celery at 30 min (D) for variant EG.5.1, showing greater persistence of the latter.

Table 1
Data of viral loads (copies/mL) and viability (TCID50/mL) of SARS-CoV-2 lineage B.1 and EG.5.1 from 0.5 h to 96 h post artificial contamination.

SARS-CoV-2 B.1 (copies/mL)							SARS-CoV-2 EG.5.1 (copies/mL)					
Apple												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	6.97	3.75	4.62E+03	1.08E+03	8.87	4.67	7.07	3.63	5.25E+03	1.08E+03	8.84	4.89
4h	6.83	2.69	2.44E+03	5.01E+02	8.67	4.81	6.93	3.60	4.62E+03	1.08E+03	8.49	4.19
8h	6.78	3.64	–	-	5.85	3.27	6.92	3.36	-	-	5.87	3.26
24h	6.75	3.47	–	–	6.09	3.26	6.89	3.64	-	-	4.37	2.19
48h	6.47	3.19	–	–	5.04	2.70	5.69	2.95	-	-	3.84	1.93
72h	6.34	3.09	–	–	4.23	2.34	5.42	2.62	-	-	–	–
96h	4.62	2.36	–	–	4.03	2.16	4.70	2.48	-	-	–	–
Grape												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	6.21	3.45	3.15E+03	7.36E+02	8.96	5.09	7.08	3.28	4.62E+03	1.08E+03	8.68	4.62
4h	6.18	3.11	1.66E+03	3.42E+02	8.92	5.05	6.80	3.41	-	-	5.71	3.05
8h	6.17	3.27	-	-	5.94	3.32	6.63	3.04	-	-	4.67	2.41
24h	6.16	3.09	-	-	4.05	2.11	4.07	2.16	-	-	4.17	2.25
48h	4.33	2.40	-	-	–	–	4.06	2.19	-	-	3.32	1.66
72h	4.25	2.28	-	-	–	–	3.94	2.20	-	-	–	–
96h	3.83	2.06	-	-	–	–	3.78	1.99	-	-	–	–
SARS-CoV-2 B.1 (copies/mL)							SARS-CoV-2 EG.5.1 (copies/mL)					
Fennel												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	6.65	3.51	7.70E+03	1.59E+03	9.01	5.09	6.76	3.05	2.24E+03	8.43E+02	8.30	4.95
4h	6.23	2.89	5.25E+03	1.08E+03	8.72	4.79	6.68	3.49	-	-	6.15	3.52
8h	5.55	3.00	-	-	5.49	2.83	6.17	3.04	-	-	5.90	3.45
24h	5.37	2.78	-	-	–	–	5.82	3.20	-	-	–	–
48h	4.26	2.29	-	-	–	–	4.25	2.10	-	-	–	–
72h	4.12	2.10	-	-	–	–	4.19	2.08	-	-	–	–
96h	3.33	1.80	-	-	–	–	3.55	1.71	-	-	–	–
Carrot												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	6.68	3.46	2.24E+03	8.43E+02	9.05	4.98	6.37	2.86	-	-	6.36	3.73
4h	6.28	3.72	1.81E+03	9.32E+02	8.77	4.86	6.34	2.73	-	-	6.15	3.45
8h	4.58	2.29	-	-	3.54	2.00	4.65	2.31	-	-	5.69	3.07
24h	4.08	2.17	-	-	–	–	4.18	2.27	-	-	–	–
48h	–	–	-	-	–	–	4.03	2.22	-	-	–	–
72h	–	–	-	-	–	–	3.55	1.86	-	-	–	–
96h	–	–	-	-	–	–	3.51	1.95	-	-	–	–
SARS-CoV-2 B.1 (copies/mL)							SARS-CoV-2 EG.5.1 (copies/mL)					
Lettuce												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	6.91	3.38	4.62E+03	1.08E+03	8.99	5.05	7.05	3.38	3.58E+03	7.36E+02	8.66	4.58
4h	6.78	3.40	2.24E+03	8.43E+02	8.66	4.52	6.99	3.53	-	-	4.72	2.75
8h	6.73	3.30	-	-	6.72	3.67	6.77	3.00	-	-	4.24	2.35
24h	6.68	3.68	-	-	4.73	2.48	6.00	3.32	-	-	3.95	2.19
48h	5.96	3.48	-	-	3.71	1.89	5.66	2.82	-	-	3.96	2.22
72h	5.73	3.03	-	-	3.15	1.73	5.29	2.58	-	-	4.12	2.25
96h	4.31	2.39	-	-	–	–	5.08	2.64	-	-	4.16	2.24
Celery												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	7.76	3.97	7.70E+04	1.59E+04	8.34	4.61	8.72	4.59	7.70E+04	1.59E+04	9.56	5.07
4h	7.30	3.92	4.62E+04	1.08E+04	8.59	4.70	8.59	3.39	-	-	4.93	2.56
8h	6.66	3.15	-	-	5.65	2.99	5.65	3.34	-	-	4.81	2.77

(*continued on next page*)

Table 1 (*continued*)

Celery												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
24h	6.10	3.34	-	-	4.74	2.44	4.74	2.93	-	-	–	–
48h	6.05	3.22	-	-	–	–	–	–	-	-	–	–
72h	4.64	2.37	-	-	–	–	–	–	-	-	–	–
96h	4.13	2.22	-	-	–	–	–	–	-	-	–	–
SARS-CoV-2 B.1 (copies/mL)							SARS-CoV-2 EG.5.1 (copies/mL)					
Tomato												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	7.44	3.98	7.70E+04	1.59E+04	9.15	5.26	8.45	4.65	4.62E+04	1.08E+04	8.79	4.99
4h	7.16	3.54	-	-	5.59	3.23	8.05	4.21	5.25E+03	1.08E+03	8.96	5.02
8h	6.60	3.52	-	-	4.90	2.67	7.94	4.21	-	-	7.12	4.00
24h	5.95	2.79	-	-	–	–	7.08	3.46	-	-	6.01	3.29
48h	5.94	3.01	-	-	–	–	6.88	3.74	-	-	4.84	2.61
72h	5.37	2.42	-	-	–	–	6.12	3.51	-	-	–	–
96h	4.86	2.87	-	-	–	–	5.58	2.87	-	-	–	–
Radish												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	8.08	4.55	7.70E+04	1.59E+04	9.18	5.19	8.55	4.39	5.25E+04	1.08E+04	8.81	4.73
4h	7.98	4.09	7.08E+03	2.67E+03	8.86	4.79	8.66	4.59	5.25E+03	1.08E+03	9.06	4.90
8h	6.80	3.63	-	-	6.53	3.47	8.29	4.41	-	-	7.15	3.82
24h	6.57	3.85	-	-	4.73	2.28	7.11	3.74	-	-	5.76	2.95
48h	5.78	2.71	-	-	–	–	6.97	3.66	-	-	–	–
72h	5.41	2.45	-	-	–	–	6.21	3.19	-	-	–	–
96h	–	–	-	-	–	–	5.85	3.20	-	-	–	–
SARS-CoV-2 B.1 (copies/mL)							SARS-CoV-2 EG.5.1 (copies/mL)					
Rocket												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	6.83	3.95	5.25E+04	1.08E+04	8.58	4.99	8.66	5.29	5.25E+03	1.08E+03	9.52	4.90
4h	7.16	4.02	5.25E+03	1.08E+03	8.63	4.62	7.20	3.78	3.91E+02	2.01E+02	9.09	4.77
8h	6.57	3.44	-	-	4.40	2.15	6.89	3.45	–	–	4.24	2.18
24h	5.68	2.92	-	-	–	–	6.73	3.54	-	-	–	–
48h	5.58	3.03	-	-	–	–	6.60	3.60	-	-	–	–
72h	–	–	-	-	–	–	6.51	3.20	-	-	–	–
96h	–	–	-	-	–	–	4.83	2.39	-	-	–	–
Cucumber												
Time (h)	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM	Swab mean (log10 copies/mL)	SEM	TCID50/mL mean (infectious particles)	TCID50/ mLSD	Cell passage mean (Log10)	SEM
0.5h	8.12	4.59	4.62E+05	1.08E+05	8.77	4.67	8.52	4.36	7.70E+03	1.59E+03	9.02	4.92
4h	7.18	3.35	-	-	5.58	2.82	7.10	3.53	1.46E+02	3.42E+01	8.88	4.87
8h	6.11	3.44	-	-	4.35	2.47	6.30	2.90	-	-	4.49	2.11
24h	5.34	2.94	-	-	–	–	5.82	2.91	-	-	–	–
48h	4.23	2.20	-	-	–	–	5.77	3.14	-	-	–	–
72h	4.15	2.18	-	-	–	–	5.59	2.58	-	-	–	–
96h	–	–	-	-	–	–	5.02	2.60	-	-	–	–

Swab: viral load analysed directly on food. at 30 min post contamination (0.5 h). 4 h. 8 h and every 24 h post contamination up to 96 h. **Cell passage**: viral load detected in the cell supernatant after 3 freezing/thawing cycles. All the procedures were performed in triplicates. Therefore. the results obtained from RT-qPCR were converted to Log10 and then SEM was calculated for each point. The association between the increase in viral load between swab UTM and cell passages. as well as the presence of cytopathic effect (CPE) on cells were used to demonstrate virus viability, followed by **TCID50/mL** detection to assess viable virus titre on the tested matrices, which is reported as a mean value and SD (standard deviation) was calculated for each timepoint.

infections revealed the presence of grape, fennel, lettuce, and celery at 30 min, whereas all the others were detectable up to 4 h and it was not detectable on carrot. Same results were obtained for the development of the CPE. Results obtained by the assessment of the cell passage and TCID50/mL were consistent with the presence of CPE among the tested foods, as shown in Fig. 5. Therefore, whenever an increase in viral loads was revealed between swab and cell passage, a viable virus was observed by TCID50 and by the observation of the CPE on cell monolayers. By contrast, when no increase in the viral loads was observed, no results were obtained by TCID50/mL and no CPE was observed on cell monolayers.

Altogether, these results appear to show that the SARS-CoV-2 lineage B.1 has longer persistence and viability than the EG.5.1 variant (Table 1).

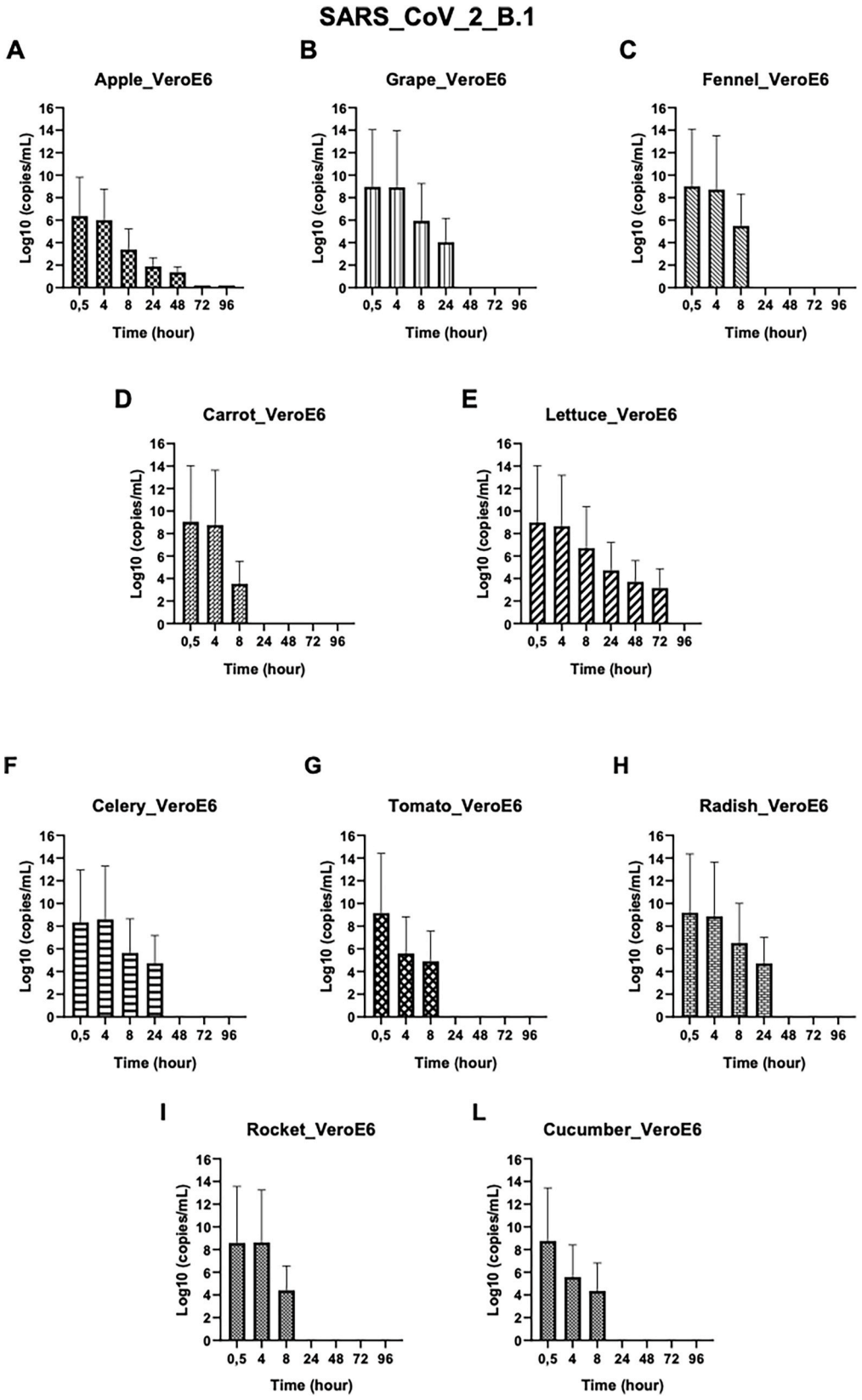


Fig. 3. Evaluation of viral RNA in the supernatant of Vero E6 cells

To assess the infectivity of SARS-CoV-2 lineage B.1, Vero E6 cells were infected with 100 μL of UTM. Viral titer was determined at 7 different sampling times and for 10 food types: apple (A), grape (B), fennel (C), carrot (D), lettuce (E), celery (F), tomato (G), radish (H), rocket (I), and cucumber (L), using the RT-qPCR technique. Viral titre reported in log10 on Y axis related to the hours of infection on X axis. After analyzing the raw data, which were $\log_{10}$ transformed, and calculating the standard error of the mean (SEM) for each point, we used GraphPad Prism to generate the corresponding graphs.

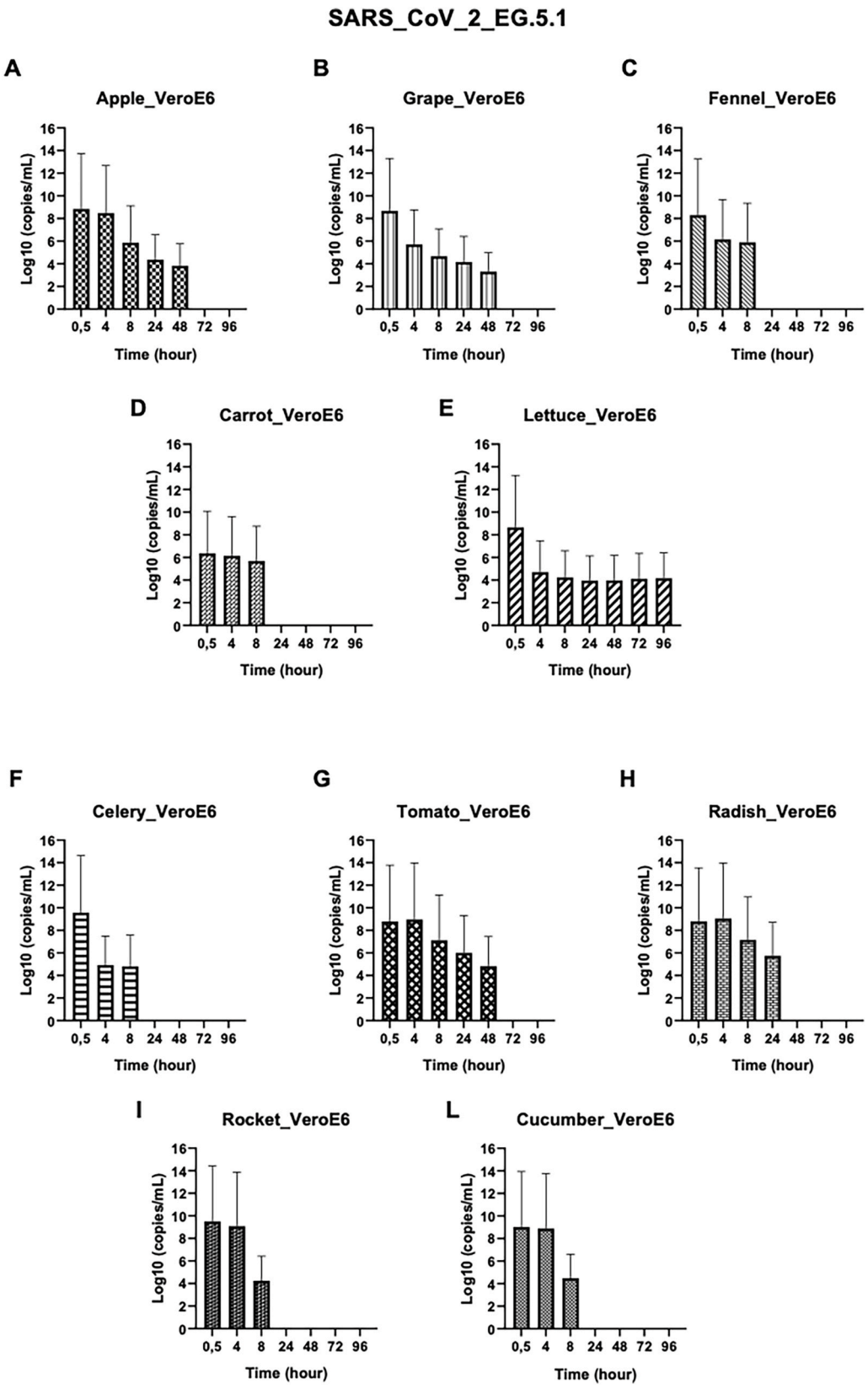
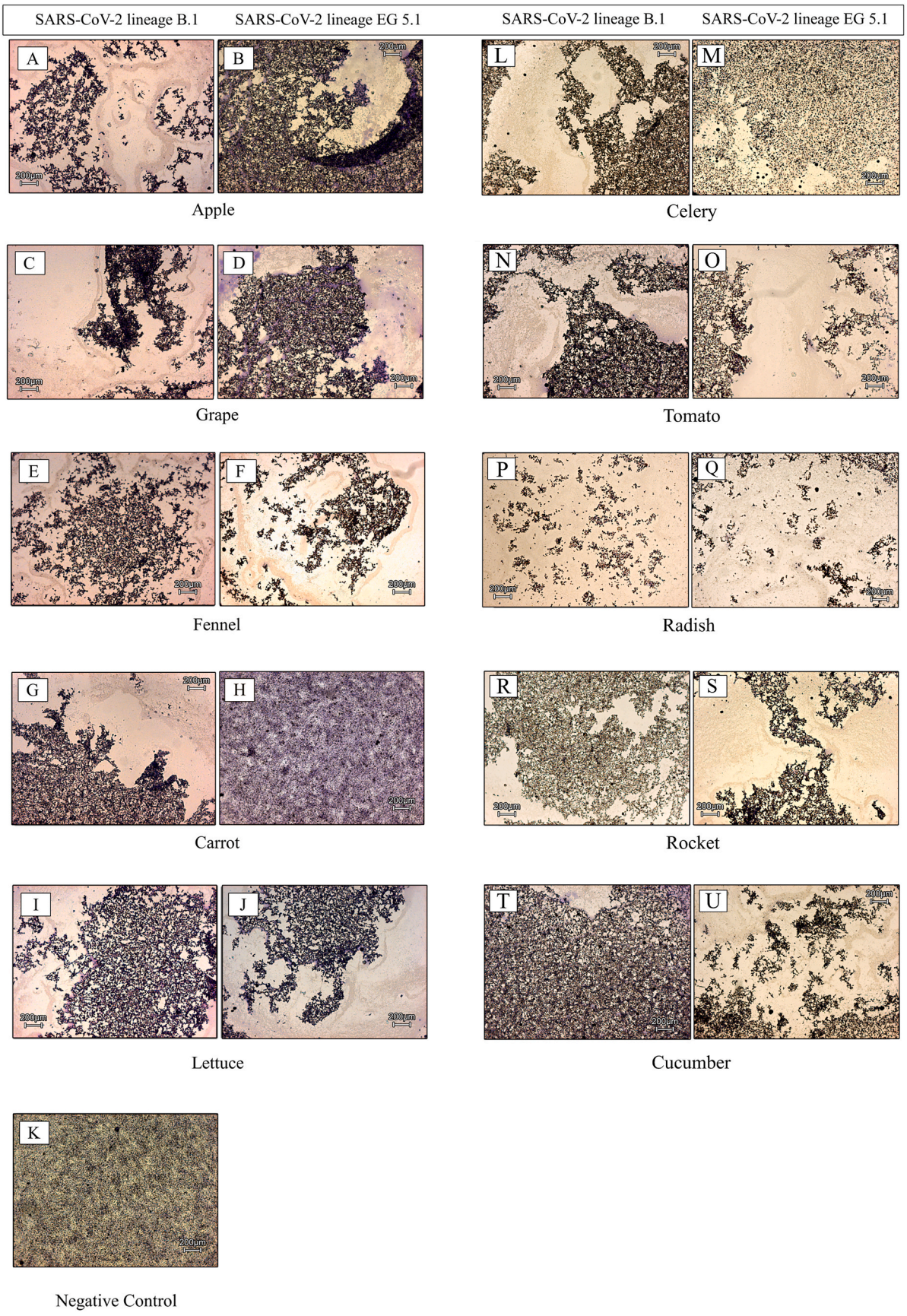


Fig. 4. Evaluation of viral RNA in the supernatant of Vero E6 cells

To assess the infectivity of SARS-CoV-2 lineage EG5.1, Vero E6 cells were infected with 100 μL of UTM. Viral titer was determined at 7 different sampling times and for 10 food types: apple (A), grape (B), fennel (C), carrot (D), lettuce (E), celery (F), tomato (G), radish (H), rocket (I), and cucumber (L), using the RT-qPCR technique. Viral titre reported in log10 on Y axis related to the hours of infection on X axis. After analyzing the raw data, which were $\log_{10}$ transformed, and calculating the standard error of the mean (SEM) for each point, we used GraphPad Prism to generate the corresponding graphs. Vero E6 infected with 100 μL of UTM collected at seven different sampling times in the contaminated plant-based food matrices with SARS-CoV-2 EG5.1 variant. The detections were performed in triplicate, so the average of the three detections was then expressed in Log10.



(caption on next page)

Fig. 5. Cytopathic effect on Vero E6 cells monolayers
Microscopy images by crystal violet staining at 5X magnification using Axiovert 25 inverted microscope (Carl Zeiss, Oberkochen, Germany) with the AxioVision 4.8 software. The cell monolayers were daily inspected every 24 h up to 7 days post inoculum to evaluate the development of any cytotoxic and cytopathic effect (CPE), induced by SARS-CoV-2 lineage B.1 and EG 5.1 on Vero E6 monolayers. A–B: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h post artificial contamination (p.c.) on apple; C–D: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h p.c. (C) and 30 min (D) p.c. on grape. E–F: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h p.c. (E) and 30 min p.c. (F) on fennel. G–H: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h p.c. for SARS-CoV-2 lineage B1 (G) and no CPE observed for the EG 5.1 variant (H) even at 30 min post contamination on carrots. I–J: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h p.c. (I) and 30 min p.c. (J) on lettuce. L–M: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h p.c. (L) and 30 min p.c. (M) on celery; N–O: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 30 min p.c. (N) and 4 h p.c. (O) on tomato; P–Q: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 4 h p.c. for both the lineages on radish; R–S: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 30 min p.c. for both the lineages on rocket; T–U: CPEs induced by the inoculum of UTM obtained on the cells, corresponding to 30 min p.c. for (T) and 4 h p.c. (U) on cucumber. The development of the CPEs was characterized by the visualization of cellular rounding, detachment, and degeneration on Vero E6 cell monolayers. K: negative control at 96 h showing an intact cell monolayer. Images obtained by inverted microscope at 5X magnification of the cytopathic effect induced by SARS-CoV-2 lineage B1 and EG 5.1 on Vero E6 monolayers.

Finally, the evaluation of the pH of the tested food matrices at different timepoints, starting at 0.5h to 96 h showed no significant pH changes over time, and no evident difference was observed between the food pH and the viability of the viruses (Table 2).

4. Discussion

To date, the role of food in the transmission of SARS-CoV-2 has not yet been fully elucidated. The determination of the different transmission routes of SARS-CoV-2, including indirect transmission, represents a key role in expanding the knowledge of the epidemiology of this virus, as this aspect is still debated and not yet fully understood. Furthermore, the implications of the continuous emergence of new variants, some of which show greater stability, virulence and pathogenicity ((ECDC, 2024)), should be assessed for the implementation of control measures in at-risk settings. Some authors have hypothesized that contaminated food and beverages may play a role in oral infection and fecal-oral transmission of SARS-CoV-2 (Chen et al., 2022; (Esseili et al., 2022); Saulnier et al., 2023; Termansen & Frische, 2023), however EFSA highlighted the absence of evidence of transmission of SARS-CoV-2 through food consumption (EFSA, 2020). On the other hand, food and food packaging can be contaminated through different routes, such as coughing/sneezing on the product, or by contact with contaminated surfaces, so if the virus remains stable on these matrices, they may act as fomites (Günther et al., 2020; Xie et al., 2020). Therefore, it is of utmost relevance to understand the viral ability to survive on food samples, especially among unprocessed foods that are consumed raw, in particular fresh fruits and vegetables. Thus, an artificial contamination of ten different fresh fruits and vegetables was performed using the SARS-CoV-2 lineage B.1 and the SARS-CoV-2 variant EG.5.1, in order to analyze whether a difference in persistence and viability could be observed both between the tested matrices and between the two lineages. Indeed, our study shows that SARS-CoV-2 EG.5.1 exhibits increased RNA stability (detectable up to 96 h) but lower viability compared to B.1 variant on plant-based foods. The hypothesis underlying the present study was the possible transmission of SARS-CoV-2 through contact with contaminated food from infected workers in the commercial, logistics or food sector involved in the transport, production, sale and consumption of plant-based foods (Günther et al., 2020; Xie et al., 2020). Other studies have evaluated the viability of SARS-CoV-2 on several food matrices ((FSA, 2022); (Esseili et al., 2022) ; Jia et al., 2022; Li et al., 2023; Tessaro et al., 2022; Jung et al., 2023; Mlinar et al., 2023; Fukuta et al., 2021), nevertheless, in the absence of a specific standard operating procedure one of the main difficulties to standardize the recovery of SARS-CoV-2 among foodstuffs, is the choice of the sampling method. Indeed, several sampling approaches have been used study recovered the virus by eluting each piece of food by inoculating viral transport media applying repeated pipetting (Li et al., 2023), other authors recovered the contaminated droplet by washing the food surface (Tessaro et al., 2022), other included pieces of food into tubes and vortexed them to recover viruses (Jung et al., 2023) or performed swabbing (Mlinar et al., 2023). Nevertheless, the Food Standard Agency (FSA) evaluated three different sampling methods, pulsification, vortexing with beads, and swabbing, to determine the best technique for recovering SARS-CoV-2 among several foodstuffs. The study concluded that, while some foods, such as cheese, sliced ham, croissants, the best recovery method was revealed to be vortexing with beads, pulsification for pepper, the swabbing method, using flocked swab, was demonstrated the best sampling method among fresh fruits (olive, apple, raspberry) and vegetables (broccoli) ((FSA, 2022)), so that, in the present study swabbing sampling was preferred to improve virus recovery.

Our findings revealed a difference in the persistence and viability of the lineages under investigation. Indeed, higher persistence was observed for the EG.5.1 variant, which was detectable in all the 10 tested food matrices for the whole experiment (up to 96 h), except for celery (up to 24 h), compared to the lineage B.1 which RNA was detectable up to 96 h post-contamination only in six foods, while lower persistence was observed in the other matrices, in particular in carrots and rockets, where it was detectable only up to 24 and 48 h, respectively. Nevertheless, PCR-based assays for detection of pathogens on foods, does not correlate well with the presence or quantity of viable virus as the virus is inactivated over time but the amount of virus RNA does not decrease in a linear manner, indicating that this method shall not be intended to evaluate the risk of persistence of residual infectious in these matrices (Jia et al., 2022). Indeed, when evaluating the viability of SARS-CoV-2, a reduced timespan was observed for the residual infectivity, with a

Table 2
Food pH evaluation at the specific sampling times for all the examined food matrices.

pH value										
Time (h)	Apple	Grape	Fennel	Carrot	Lettuce	Celery	Tomato	Radish	Rocket	Cucumber
0.5	3.9	3.8	5.8	5.6	5.8	5.8	4.2	2.8	6	5.5
4	4.2	3.8	6	5.8	6	6	4.5	3.2	6	5.5
8	4.2	4	6	5.6	6	6.2	4.6	3	6.2	5.5
24	4.5	4	6	5.6	6	6	4.5	3	6.2	5
48	4.5	4	6	5.8	6	6	4.5	3	6	5
72	4.5	4	6.2	5.8	6	6	4.5	3	6.3	5
96	4.2	4.5	6.2	5.8	6.2	6.3	4.8	3	6.4	5

maximum survival time of 4 h. In the present study the viability of the virus was demonstrated through three main steps, i) a slight rise in viral loads between the swab and the cell passage by RT-qPCR, indicating that the virus was still viable and able to replicate, ii) the development of the CPE as well as iii) virus titration by TCID50. As demonstrated for virus RNA persistence, also in this case a difference was revealed between the two lineages and between the different matrices. Indeed, longer viability was observed for SARS-CoV-2 lineage B.1, which was still viable at 4 h post inoculum onto the cell monolayers among eight foods, while among tomato and cucumber it was viable only for 30 min. Conversely, SARS-CoV-2 EG.5.1 variant showed in most cases a viability for 30 min, 4 h only for apple, tomato, radish, rocket, and cucumber, while on carrot as soon as time 0 it was not demonstrated either CPE or residual infectivity. This complex scenario also reflects what has been done so far in the literature, where various explanations have been proposed for the persistence of the virus on different types of food. Tomato, as well as grapes and lettuce have already been studied, even though with different strains from those used in our research. For instance, some studies have suggested that phenolic compounds in grape skins may inactivate the virus by binding to it, thus exhibiting antiviral properties (Hudson, 2018). However, more recent studies have shown that the viral concentration on the surface of intact grapes did not significantly decrease within seven days (Jia et al., 2022). Similarly, on tomato surface the virus was still detectable for up to 24 h or up to 21 days at 4 °C (Jia et al., 2022). In contrast, different results were revealed among lettuce, which was already analysed by Jung and colleagues (Jung et al., 2023). The authors observed a maximum survival time of three days, whereas our findings showed a viability time of 4 h and 30 min p.c. for the B.1 and EG.5.1 lineages, respectively, and RNA persistence observed up to 96 h p.c. for both lineages. The differences found on the vitality of the virus between our results and those already reported in the literature on the same matrices may be ascribed to various aspects. Certainly, a preponderant aspect that influences the viability on a food matrix is the temperature effect in preserving the infectivity of the virus, as many authors have used a refrigeration temperature of 4 °C compared to the present study in which room temperature at 22.5 °C was used. In this context, longer viability has been observed also in several other studies involving aerosol, common surfaces, and food matrices, where an increasing survival is observed with decreasing temperature and relative humidity ((Esseili et al., 2022) ; Jung et al., 2023; Lelieveld et al., 2020; Riddell, Goldie, Hill, Eagles, & Drew, 2020). Esseili et al. (2022) observed a wide difference in the infectivity over time between SARS-CoV-2-inoculated berries stored at 4 °C and at freeze temperature. Indeed, while at refrigerated temperature the viability of the virus was assessed up to 7 days, and a significant reduction in the infectivity was observed over time, at freezing temperature infectious SARS-CoV-2 was recovered for 28 days without significant reductions. Moreover, another critical factor in virus viability is the pH of the food matrix. SARS-CoV-2 is stable at pH levels between 3 and 10 at room temperature, with more extreme acidic or alkaline conditions leading to viral inactivation (Chin et al., 2020). For instance, foods with a lower pH (like strawberries, tomatoes, and apples) tend to inactivate the virus faster compared to less acidic foods (such as cucumbers and lettuce) (Anelich et al., 2020; Blondin-Brosseau et al., 2021; Vitucci et al., 2021). However, the specific viral lineage may also influence survival more than intrinsic food characteristics. Despite it, in our study the pH evaluation, on each food on the 10 plant-based food test, no significant changes were observed between 30 min p.c. and the end of the experiment, as well as no evident difference was observed between the food pH and the viability of the viruses. Therefore, it is possible that the factor mostly affecting the persistence and viability of SARS-CoV-2 was the different lineage or to other environmental characteristics than intrinsic food pH. Indeed, it has been observed that the ability of a virus to remain infectious ex vivo depends largely on environmental conditions. Indeed several intrinsic and extrinsic factors, both of the virus and of the surface, have been identified to be able to impact on virus persistence and survival, such as droplet size, environmental conditions, as humidity, temperature and UV exposure, as well as virus species and strain, in particular by intrinsic chemical-physical properties, such as the absorption capacity of the matrices as well as the presence of antiviral molecules, which could partially explain the differences in persistence and vitality observed in our study on different matrices. Indeed, the reabsorption of the medium of the droplets may lead to a rapid deterioration of the microenvironment, impairing the ability to persist and survive on a matrix (Aboubakr et al., 2021; Biryukov et al., 2020; Chin et al., 2020; Fusco et al., 2023; Guo et al., 2020; Kwon et al., 2021). Furthermore, it has been proven that longer survival is determined by the presence of antiviral properties of such types of foods, in particular meats and seafood compared to fruit and vegetables, as proteins and fats may stabilize the virus and maintain the infectivity (Dhakal et al., 2021).

Our findings, as well as those discussed in several studies in literature (Glasbrenner et al., 2023; Li et al., 2022, 2023) highlight the importance of considering the risk of contact with potentially infected foods, especially if consumed raw and unprocessed. Although there is no certainty about the possible absorption of the virus via the enteric route, the main risk could arise from contact with a contaminated foodstuff, which can act a fomite, thus favoring the transmission of SARS-CoV-2 via the contact to the hands and subsequently to mucous membranes of the mouth, nose, or eyes (Geng & Wang, 2023).

A major limitation of this study lies in the ongoing uncertainty surrounding the minimum infectious dose of SARS-CoV-2 required to cause human infection, as existing data are predominantly derived from observational human studies. SeyedAlinaghi et al. (2022) conducted a systematic review reporting that the infectious dose responsible for disease in humans can be as low as 1.26 plaque-forming units (PFU) and may reach up to $7 \times 10^{6.25}$ PFU. However, accurately identifying the true infectious dose, or quantifying the real risk of transmission via fomites, remains challenging (Geng & Wang, 2023). Together with several other investigations (FSA, 2022), our results indicate that the risk of SARS-CoV-2 transmission through contaminated plant-based foods cannot be considered negligible, particularly within the first few hours after contamination. This concern is amplified in food retail environments or open markets, where unpackaged fruits and vegetables are sold and typically consumed raw. However, although the persistence of viral RNA indicates a possible risk of fomite transmission, the limited viability period ($\leq$4 h) reduces the chances of transmission through contaminated food.

Although we endeavored to reproduce realistic conditions in the laboratory by using a 50 μL inoculum at 10^5 TCID50/mL, a concentration approximating viral loads observed in the upper respiratory tract of infected individuals (Karimzadeh et al., 2021; Xue et al., 2020), as well as maintaining standard indoor temperature and relative humidity, our experimental setup does not constitute a direct measure of fomite-based exposure risk. Indeed, artificial contamination may not perfectly reproduce real-life exposure scenarios, such as differences in droplet size or environmental conditions. However, these findings should be viewed as a reference point for managing risk in closed or densely populated settings (Fusco et al., 2023). and for everyday touched foodstuff that might become contaminated.

Another limit of the study may be identified in the evaluation of only two lineages. Nevertheless, the primary aim of this study was to trace the evolutionary shift in SARS-CoV-2 environmental stability by comparing the wild-type strain with one of widely circulating omicron-subvariant in late 2023 (WHO, 2023b; ISS, 2023), to directly measure how intrinsic viral changes may affect virus ability to remain infectious on plant-based food surfaces. Therefore, the main goal was not to catalogue the latest circulating lineages; rather, to demonstrate that viral evolution can meaningfully alter the risk posed by contaminated food matrices. Specifically, we used the wild-type virus as a baseline and compared it with one of the representatives of the omicron-sub lineages, so that any observed differences in survival or decay rates could be attributed to intrinsic evolutionary adaptations. Our data shows that, despite

exhibiting higher transmissibility or clinical severity, some variants may possess greater environmental stability than the original strain, bus showing lower viability than their predecessor. Such findings elucidate general trends in coronavirus evolution, which may prove invaluable for future studies. Indeed, when SARS-CoV-2 first emerged, knowledge gained from SARS and MERS was used to obtain early risk assessments (Zhang et al., 2020; Zhou et al., 2021; Zhu et al., 2020).

It is also important to acknowledge that, since the World Health Organization downgraded COVID-19 from a "public health emergency of international concern" over two years ago (WHO, 2023a), official surveillance of SARS-CoV-2 has been scaled back in many regions. Laboratories no longer routinely isolate or archive the newest variants, as the global emphasis has shifted toward endemic management rather than emergency response. These practical constraints made it difficult to obtain sufficient viral titers of currently circulating lineages for rigorous stability experiments. Nevertheless, our results clearly demonstrate the impact of viral evolution on environmental stability without confounding influences from minor or rapidly shifting sub-lineages. In light of these considerations, we believe our findings remain highly relevant for ongoing risk assessments of plant-based foods as potential fomites, even as new variants continue to emerge.

5. Conclusions

Several factors influence SARS-CoV-2 viability, in particular temperature and relative humidity. Low temperatures, in fact, play a key role in increasing the persistence of the virus. In the food industry, clarifying the correlation between the intrinsic characteristics of food and the viability of the different SARS-CoV-2 variants is of major importance in order to have a good level of awareness for food-workers that, through accidental self-inoculation, can be exposed to the infection. Moreover, infected operators in the food retail, catering, or cold-chain industry, may spread the virus through contamination of foods, which is then exposed to consumers, or other operators. Our results have underlined that both SARS-CoV-2 lineage B.1 and SARS-CoV-2 EG.5.1 variant are able to persist and survive on certain plant foods, thus they may represent a possible source of virus transmission. Therefore, this work highlights the importance of plant-based food fomites in demonstrating the varying infectious capacities of the different strains. Future studies may elucidate on the probable link between environmental and/or chemical parameters of other type of foods and the survivability of the different SARS-CoV-2 variants, also considering their structural and functional mutations. Furthermore, our findings highlight the impact of virus strains in the virus stability between and importance of the emergence of novel circulating variants, as already demonstrated on inanimate surfaces (Fusco et al., 2023).

CRediT authorship contribution statement

Giovanna Fusco: Writing – review & editing, Supervision, Conceptualization. **Gerardo Picazio:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Sergio Brandi:** Methodology, Investigation, Formal analysis. **Lorena Cardillo:** Writing – original draft. **Loredana Cozzolino:** Methodology, Investigation. **Maurizio Viscardi:** Methodology, Investigation, Formal analysis. **Alessia Pucciarelli:** Methodology, Investigation, Formal analysis, Data curation. **Federica Di Maggio:** Writing – original draft, Formal analysis, Data curation. **Marcella Nunziato:** Writing – original draft, Formal analysis, Data curation. **Esterina De Carlo:** Writing – review & editing, Supervision, Conceptualization. **Claudio de Martinis:** Writing – original draft, Data curation. **Francesco Salvatore:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Disclosure statement

The authors declare no potential conflict of interest.

Ethic statement

For the content of the study, no ethical approval was necessary.

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Declaration of competing interest

The authors declare no potential conflict of interest.

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Data availability

All data supporting the study are reported within the manuscript

References

Aboubakr, H. A., Sharafeldin, T. A., & Goyal, S. M. (2021). Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: A review. *Transboundary and Emerging Diseases, 68* (2), 296–312. https://doi.org/10.1111/tbed.13707

Anelich, L. E. C. M., Lues, R., Farber, J. M., & Parreira, V. R. (2020). SARS-CoV-2 and risk to food safety. *Frontiers in Nutrition, 7*, Article 580551. https://doi.org/10.3389/fnut.2020.580551

Arienzo, A., Gallo, V., Tomassetti, F., Pitaro, N., Pitaro, M., & Antonini, G. (2023). A narrative review of alternative transmission routes of COVID 19: What we know so far. *Pathogens and Global Health, 117*(8), 681–695. https://doi.org/10.1080/20477724.2023.2228048

Bai, L., Wang, Y., Wang, Y., Wu, Y., Li, N., & Liu, Z. (2021). Controlling COVID-19 transmission due to contaminated imported frozen food and food packaging. *China CDC weekly, 3*(2), 30–33. https://doi.org/10.46234/ccdcw2021.008

Biryukov, J., Boydston, J. A., Dunning, R. A., Yeager, J. J., Wood, S., Reese, A. L., Ferris, A., Miller, D., Weaver, W., Zeitouni, N. E., Phillips, A., Freeburger, D., Hooper, I., Ratnesar-Shumate, S., Yolitz, J., Krause, M., Williams, G., Dawson, D. G., Herzog, A., … Altamura, L. A. (2020). Increasing temperature and relative humidity accelerates inactivation of SARS-CoV-2 on surfaces. *mSphere, 5*(4), Article e00441. https://doi.org/10.1128/mSphere.00441-20, 20.

Blondin-Brosseau, M., Harlow, J., Doctor, T., & Nasheri, N. (2021). Examining the persistence of human coronavirus 229E on fresh produce. *Food Microbiology, 98*, Article 103780. https://doi.org/10.1016/j.fm.2021.103780

Cardillo, L., de Martinis, C., Viscardi, M., Esposito, C., Sannino, E., Lucibelli, G., Limone, A., Pellino, S., Anastasio, R., Pellicanò, R., Baldi, L., Galiero, G., & Fusco, G. (2021). SARS-CoV-2 quantitative real time PCR and viral loads analysis among asymptomatic and symptomatic patients: An observational study on an outbreak in two nursing facilities in Campania region (southern Italy). *Infectious Agents and Cancer, 16*(1), 45. https://doi.org/10.1186/s13027-021-00388-x

Centers for Disease Control and Prevention (CDC). Science brief: SARS-CoV-2 and surface (fomite) transmission for indoor community environments. Retrieved from: https://archive.cdc.gov/#/details?url=https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/surface-transmission.html. Accessed May 25, 2025.

Céspedes-Tenorio, D., & Arias-Arias, J. L. (2023). The virus-induced cytopathic effect. *Subcellular Biochemistry, 106*, 197–210. https://doi.org/10.1007/978-3-031-40086-5_7

Cevik, M., Kuppalli, K., Kindrachuk, J., & Peiris, M. (2020). Virology, transmission, and pathogenesis of SARS-CoV-2. *BMJ, 371*, Article m3862. https://doi.org/10.1136/bmj.m3862

Ceylan, Z., Meral, R., & Cetinkaya, T. (2020). Relevance of SARS-CoV-2 in food safety and food hygiene: Potential preventive measures, suggestions and nanotechnological

approaches. *Virus disease, 31*(2), 154–160. https://doi.org/10.1007/s13337-020-00611-0
Chen, T. H., Hsu, M. T., Lee, M. Y., & Chou, C. K. (2022). Gastrointestinal involvement in SARS-CoV-2 infection. *Viruses, 14*(6), 1188. https://doi.org/10.3390/v14061188
Chin, A. W. H., Chu, J. T. S., Perera, M. R. A., Hui, K. P. Y., Yen, H. L., Chan, M. C. W., Peiris, M., & Poon, L. L. M. (2020). Stability of SARS-CoV-2 in different environmental conditions. *The Lancet Microbe, 1*(1), e10. https://doi.org/10.1016/S2666-5247(20)30003-3
D'Argenio, V., Borrillo, F., Cariati, F., Di Maggio, F., & Tomaiuolo, R. (2019). Glossary of molecular biology and clinical molecular biology. Part I: General terms. *Biochimica Clinica, 43*, 90–105. https://doi.org/10.19186/BC_2019.008
De Rose, D. U., Reposi, M. P., Amadio, P., Auriti, C., Dall'Oglio, I., Corsetti, T., Dotta, A., & Salvatori, G. (2020). Use of disinfectant wipes to sanitize milk's containers of human milk bank during COVID-19 pandemic. *Journal of Human Lactation: Official Journal of International Lactation Consultant Association, 36*(3), 547–549. https://doi.org/10.1177/0890334420924639
Dhakal, J., Jia, M., Joyce, J. D., Moore, G. A., Ovissipour, R., & Bertke, A. S. (2021). Survival of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Herpes simplex virus 1 (HSV-1) on foods stored at refrigerated temperature. *Foods, 10*(5), 1005. https://doi.org/10.3390/foods10051005
Dyal, J. W., Grant, M. P., Broadwater, K., Bjork, A., Waltenburg, M. A., Gibbins, J. D., Hale, C., Silver, M., Fischer, M., Steinberg, J., Basler, C. A., Jacobs, J. R., Kennedy, E. D., Tomasi, S., Trout, D., Hornsby-Myers, J., Oussayef, N. L., Delaney, L. J., Patel, K., Shetty, V., … Honein, M. A. (2020). COVID-19 among workers in meat and poultry processing facilities - 19 states, April 2020. *MMWR. Morbidity and mortality weekly report, 69*(18). https://doi.org/10.15585/mmwr.mm6918e3, 10.15585/mmwr.mm6918e3.
Economou, V., Sakkas, H., Bezirtzoglou, E., Papa, A., & Soultos, N. (2021). SARS–CoV–2 and food—how confident are we about them? *Hygie, 1*, 80–98. https://doi.org/10.3390/hygiene1030008
Esseili, M. A., Mann, A., Narwankar, R., Kassem, I. I., Diez-Gonzalez, F., & Hogan, R. J. (2022). SARS-CoV-2 remains infectious for at least a month on artificially-contaminated frozen berries. *Food Microbiology, 107*, Article 104084. https://doi.org/10.1016/j.fm.2022.104084
European Centre for Disease Prevention and Control (ECDC). (2024). SARS-CoV-2 variants of concern as of 30 August 2024. Retrieved from https://www.ecdc.europa.eu/en/covid-19/variants-concern. (Accessed 3 March 2025).
European Food Safety Authority (EFSA). (2020). Coronavirus: No evidence that food is a source or transmission route. Retrieved from http://www.efsa.europa.eu/en/news/coronavirus-no-evidence-food-source-ortransmission-route. (Accessed 15 July 2024).
Food Standard Agency (FSA). (2022). Survival of SARS-CoV-2 on the surfaces of food and food packaging materials. Retrieved from https://www.food.gov.uk/research/survival-of-sars-cov-2-on-food-surfaces-abbreviations-and-codes. (Accessed 28 February 2025) https://doi.org/10.46756/sci.fsa.kww583.
Fukuta, M., Mao, Z. Q., Morita, K., & Moi, M. L. (2021). Stability and infectivity of SARS-CoV-2 and viral RNA in water, commercial beverages, and bodily fluids. *Frontiers in Microbiology, 12*, Article 667956. https://doi.org/10.3389/fmicb.2021.667956
Fusco, G., Picazio, G., Cardillo, L., Pucciarelli, A., Marati, L., Di Maggio, F., Nunziato, M., Brandi, S., De Carlo, E., de Martinis, C., & Salvatore, F. (2023). A comparative study on the persistence and viability of SARS-CoV-2 wild-type and omicron variant on artificially contaminated surfaces: The role of fomites. *Emerging Microbes & Infections, 12*(2), Article 2239941. https://doi.org/10.1080/22221751.2023.2239941
Geng, Y., & Wang, Y. (2023). Stability and transmissibility of SARS-CoV-2 in the environment. *Journal of Medical Virology, 95*(1), Article e28103. https://doi.org/10.1002/jmv.28103
Giobbe, G. G., Bonfante, F., Jones, B. C., Gagliano, O., Luni, C., Zambaiti, E., Perin, S., Laterza, C., Busslinger, G., Stuart, H., Pagliari, M., Bortolami, A., Mazzetto, E., Manfredi, A., Colantuono, C., Di Filippo, L., Pellegata, A. F., Panzarin, V., Thapar, N., Li, V. S. W., … De Coppi, P. (2021). SARS-CoV-2 infection and replication in human gastric organoids. *Nature Communications, 12*(1), 6610. https://doi.org/10.1038/s41467-021-26762-2
Glasbrenner, D. C., Choi, Y. W., & Middleton, J. K. (2023). SARS-CoV-2 persistence on common food covering materials: Plastic wrap, fruit wax, and cardboard takeout containers. *Journal of Applied Microbiology, 134*(2), lxac071. https://doi.org/10.1093/jambio/lxac071
Godoy, M. G., Kibenge, M. J. T., & Kibenge, F. S. B. (2021). SARS-CoV-2 transmission via aquatic food animal species or their products: A review. *Aquaculture, 536*, Article 736460. https://doi.org/10.1016/j.aquaculture.2021.736460
Günther, T., Czech-Sioli, M., Indenbirken, D., Robitaille, A., Tenhaken, P., Exner, M., Ottinger, M., Fischer, N., Grundhoff, A., & Brinkmann, M. M. (2020). SARS-CoV-2 outbreak investigation in a German meat processing plant. *EMBO Molecular Medicine, 12*(12), Article e13296. https://doi.org/10.15252/emmm.202013296
Guo, Z. D., Wang, Z. Y., Zhang, S. F., Li, X., Li, L., Li, C., Cui, Y., Fu, R. B., Dong, Y. Z., Chi, X. Y., Zhang, M. Y., Liu, K., Cao, C., Liu, B., Zhang, K., Gao, Y. W., Lu, B., & Chen, W. (2020). Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, wuhan, China, 2020. *Emerging Infectious Diseases, 26*(7), 1583–1591. https://doi.org/10.3201/eid2607.200885
Han, J., Zhang, X., He, S., & Jia, P. (2021). Can the coronavirus disease be transmitted from food? A review of evidence, risks, policies and knowledge gaps. *Environmental Chemistry Letters, 19*(1), 5–16. https://doi.org/10.1007/s10311-020-01101-x
Hoffmann, D. (2023). The role of the oral cavity in SARS-CoV-2- and other viral infections. *Clinical Oral Investigations, 27*(Suppl 1), 15–22. https://doi.org/10.1007/s00784-023-05078-z
Hudson, J. B. (2018). *Antiviral compounds from plants* (1st ed.). Boca Raton, FL, USA: CRC Press.
Istituto Superiore di Sanità (ISS). Stima della prevalenza delle principali varianti del virus SARS-CoV-2 circolanti in Italia (novembre 2023). Retrieved from: https://www.iss.it/documents/20126/0/Relazione+tecnica+Stima+di+prevalenza+delle+varianti+del+virus+SARS+CoV2+novembre.pdf/e2384744-c38a-8f1c-71fd-6ebf3b9f5ac2. Accessed June 4, 2025.
Jia, M., Taylor, T. M., Senger, S. M., Ovissipour, R., & Bertke, A. S. (2022). SARS-CoV-2 remains infectious on refrigerated deli food, meats, and fresh produce for up to 21 days. *Foods, 11*(3), 286. https://doi.org/10.3390/foods11030286
Jung, S., Yeo, D., Wang, Z., Woo, S., Seo, Y., Hossain, M. I., & Choi, C. (2023). Viability of SARS-CoV-2 on lettuce, chicken, and salmon and its inactivation by peracetic acid, ethanol, and chlorine dioxide. *Food Microbiology, 110*, Article 104164. https://doi.org/10.1016/j.fm.2022.104164
Kärber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie, 162*, 480–483.
Karimzadeh, S., Bhopal, R., & Nguyen Tien, H. (2021). Review of infective dose, routes of transmission and outcome of COVID-19 caused by the SARS-COV-2: Comparison with other respiratory viruses. *Epidemiology and Infection, 149*, e96. https://doi.org/10.1017/S0950268821000790
Kwon, T., Gaudreault, N. N., & Richt, J. A. (2021). Environmental stability of SARS-CoV-2 on different types of surfaces under indoor and seasonal climate conditions. *Pathogens, 10*(2), 227. https://doi.org/10.3390/pathogens10020227
Le Guyader, F. S., Le Saux, J. C., Ambert-Balay, K., Krol, J., Serais, O., Parnaudeau, S., Giraudon, H., Delmas, G., Pommepuy, M., Pothier, P., & Atmar, R. L. (2008). Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *Journal of Clinical Microbiology, 46*(12), 4011–4017. https://doi.org/10.1128/JCM.01044-08
Lelieveld, J., Helleis, F., Borrmann, S., Cheng, Y., Drewnick, F., Haug, G., Klimach, T., Sciare, J., Su, H., & Pöschl, U. (2020). Model calculations of aerosol transmission and infection risk of COVID-19 in indoor environments. *International Journal of Environmental Research and Public Health, 17*(21), 8114. https://doi.org/10.3390/ijerph17218114
Li, Y., Qiao, J., Han, X., Zhao, Z., Kou, J., Zhang, W., Man, S., & Ma, L. (2022). Needs, challenges and countermeasures of SARS-CoV-2 surveillance in cold-chain foods and packaging to prevent possible COVID-19 resurgence: A perspective from advanced detections. *Viruses, 15*(1), 120. https://doi.org/10.3390/v15010120
Li, F., Xu, K., Pan, Y., Liu, P., Zhang, J., Yang, M., Lei, W., Feng, Z., Liang, Z., Zhang, D., Wu, G., & Wang, Q. (2023). Stability of SARS-CoV-2 and persistence of viral nucleic acids on common foods and widely used packaging material surfaces. *Journal of Medical Virology, 95*(6), Article e28871. https://doi.org/10.1002/jmv.28871
Liu, P., Yang, M., Zhao, X., Guo, Y., Wang, L., Zhang, J., Lei, W., Han, W., Jiang, F., Liu, W. J., Gao, G. F., & Wu, G. (2020). Cold-chain transportation in the frozen food industry May have caused a recurrence of COVID-19 cases in destination: Successful isolation of SARS-CoV-2 virus from the imported frozen cod package surface. *Biosafety and health, 2*(4), 199–201. https://doi.org/10.1016/j.bsheal.2020.11.003
Meyerowitz, E. A., Richterman, A., Gandhi, R. T., & Sax, P. E. (2021). Transmission of SARS-CoV-2: A review of viral, host, and environmental factors. *Annals of Internal Medicine, 174*(1), 69–79. https://doi.org/10.7326/M20-5008
Minami, S., Matsumoto, N., Omori, H., Nakamura, Y., Tamiya, S., Nouda, R., Nurdin, J. A., Yamasaki, M., Kotaki, T., Kanai, Y., Okamoto, T., Tachibana, T., Ushijima, H., Kobayashi, T., & Sato, S. (2023). Effective SARS-CoV-2 replication of monolayers of intestinal epithelial cells differentiated from human induced pluripotent stem cells. *Scientific Reports, 13*(1), Article 11610. https://doi.org/10.1038/s41598-023-38548-1
Mlinar, Z., Kostelac, D., Kovaček, I., Klobučar, A., Tešić, V., Prahin, V., & Frece, J. (2023). Investigation of SARS-CoV-2 detection method applicability and virus occurrence in food and food packaging. *Food Technology and Biotechnology, 61*(2), 250–258. https://doi.org/10.17113/ftb.61.02.23.8018
Nakat, Z., & Bou-Mitri, C. (2021). COVID-19 and the food industry: Readiness assessment. *Food Control, 121*, Article 107661. https://doi.org/10.1016/j.foodcont.2020.107661
Pang, X., Ren, L., Wu, S., Ma, W., Yang, J., Di, L., Li, J., Xiao, Y., Kang, L., Du, S., Du, J., Wang, J., Li, G., Zhai, S., Chen, L., Zhou, W., Lai, S., Gao, L., Pan, Y., Wang, Q., … COVID-19 Laboratory Testing Group. (2020). Cold-chain food contamination as the possible origin of COVID-19 resurgence in beijing. *National Science Review, 7*(12), 1861–1864. https://doi.org/10.1093/nsr/nwaa264
Pressman, P., Naidu, A. S., & Clemens, R. (2020). COVID-19 and food safety: Risk management and future considerations. *Nutrition Today, 55*, 125–128. https://doi.org/10.1097/NT.0000000000000415
Riddell, S., Goldie, S., Hill, A., Eagles, D., & Drew, T. W. (2020). The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *Virology Journal, 17*(1), 145. https://doi.org/10.1186/s12985-020-01418-7
Saulnier, A., Wendling, J. M., Hermant, B., & Lepelletier, D. (2023). SARS-CoV-2 transmission modes: Why and how contamination occurs around shared meals and drinks? *Food Microbiology, 114*, Article 104297. https://doi.org/10.1016/j.fm.2023.104297
SeyedAlinaghi, S., Karimi, A., Mojdeganlou, H., Pashaei, Z., Mirzapour, P., Shamsabadi, A., Barzegary, A., Afroughi, F., Dehghani, S., Janfaza, N., Fakhfouri, A., Khodaei, S., Mehraeen, E., & Dadras, O. (2022). *Minimum infective dose of severe acute respiratory syndrome coronavirus 2 based on the current evidence: A systematic review* (Vol. 10). SAGE open medicine, Article 20503121221115053. https://doi.org/10.1177/20503121221115053
Shirbhate, E., Pandey, J., Patel, V. K., Kamal, M., Jawaid, T., Gorain, B., Kesharwani, P., & Rajak, H. (2021). Understanding the role of ACE-2 receptor in pathogenesis of

COVID-19 disease: A potential approach for therapeutic intervention. *Pharmacological Reports: PR, 73*(6), 1539–1550. https://doi.org/10.1007/s43440-021-00303-6

Stals, A., Baert, L., Van Coillie, E., & Uyttendaele, M. (2012). Extraction of food-borne viruses from food samples: A review. *International Journal of Food Microbiology, 153* (1–2), 1–9. https://doi.org/10.1016/j.ijfoodmicro.2011.10.014

Termansen, M. B., & Frische, S. (2023). Fecal-oral transmission of SARS-CoV-2: A systematic review of evidence from epidemiological and experimental studies. *American Journal of Infection Control, 51*(12), 1430–1437. https://doi.org/10.1016/j.ajic.2023.04.170

Tessaro, L., Aquino, A., Panzenhagen, P., Ochioni, A. C., Mutz, Y. S., Raymundo-Pereira, P. A., Vieira, I. R. S., Belem, N. K. R., & Conte-Junior, C. A. (2022). Development and application of an SPR nanobiosensor based on AuNPs for the detection of SARS-CoV-2 on food surfaces. *Biosensors, 12*(12), 1101. https://doi.org/10.3390/bios12121101

United States Department of Agriculture (USDA). (2020). Coronavirus disease (COVID-19). Retrieved from https://www.usda.gov/coronavirus. (Accessed 25 June 2024).

van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., Tamin, A., Harcourt, J. L., Thornburg, N. J., Gerber, S. I., Lloyd-Smith, J. O., de Wit, E., & Munster, V. J. (2020). Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *New England Journal of Medicine, 382* (16), 1564–1567. https://doi.org/10.1056/NEJMc2004973

Vitucci, D., Amoresano, A., Nunziato, M., Muoio, S., Alfieri, A., Oriani, G., Scalfi, L., Frusciante, L., Rigano, M. M., Pucci, P., Fontana, L., Buono, P., & Salvatore, F. (2021). Nutritional controlled preparation and administration of different tomato purées indicate increase of β-Carotene and lycopene isoforms, and of antioxidant potential in human blood bioavailability: A pilot study. *Nutrients, 13*(4), 1336. https://doi.org/10.3390/nu13041336

Warnes, S. L., Little, Z. R., & Keevil, C. W. (2015). Human coronavirus 229E remains infectious on common touch surface materials. *mBio, 6*(6), Article e01697. https://doi.org/10.1128/mBio.01697-15, 15.

World Health Organization (WHO). (2023a). Statement on the fifteenth meeting of the IHR (2005) emergency committee on the COVID-19 pandemic. Retrieved from https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic. (Accessed 26 May 2025).

World Health Organization (WHO). (2023b). Executive summary updated risk evaluation for EG.5 and its sublineages, 21 November 2023. Retrieved from https://www.who.int/docs/default-source/coronaviruse/21112023_eg.5_ure.pdf?sfvrsn=35d6cf7d_1. (Accessed 4 June 2025).

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., & Shan, H. (2020). Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology, 158*(6), 1831–1833.e3. https://doi.org/10.1053/j.gastro.2020.02.055

Xie, C., Zhao, H., Li, K., Zhang, Z., Lu, X., Peng, H., Wang, D., Chen, J., Zhang, X., Wu, D., Gu, Y., Yuan, J., Zhang, L., & Lu, J. (2020). The evidence of indirect transmission of SARS-CoV-2 reported in guangzhou, China. *BMC Public Health, 20*(1), 1202. https://doi.org/10.1186/s12889-020-09296-y

Xue, X., Ball, J. K., Alexander, C., & Alexander, M. R. (2020). All surfaces are not equal in contact transmission of SARS-CoV-2. *Matter, 3*(5), 1433–1441. https://doi.org/10.1016/j.matt.2020.10.006

Yekta, R., Vahid-Dastjerdi, L., Norouzbeigi, S., & Mortazavian, A. M. (2021). Food products as potential carriers of SARS-CoV-2. *Food Control, 123*, Article 107754. https://doi.org/10.1016/j.foodcont.2020.107754

Zhan, J. (2020). Frozen south American white shrimp products from pingxiang, Jiangxi, tested positive for nucleic acid in their outer packaging. Retrieved from http://www.xinhuanet.com/politics/2020-07/15/c_1126239771.htm. (Accessed 25 July 2024).

Zhang, G., Hu, C., Luo, L., Fang, F., Chen, Y., Li, J., Peng, Z., & Pan, H. (2020). Clinical features and short-term outcomes of 221 patients with COVID-19 in wuhan, China. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology, 127*, Article 104364. https://doi.org/10.1016/j.jcv.2020.104364

Zhou, H., Yang, J., Zhou, C., Chen, B., Fang, H., Chen, S., Zhang, X., Wang, L., & Zhang, L. (2021). A review of SARS-CoV2: Compared with SARS-CoV and MERS-CoV. *Frontiers of Medicine, 8*, Article 628370. https://doi.org/10.3389/fmed.2021.628370

Zhu, Z., Lian, X., Su, X., Wu, W., Marraro, G. A., & Zeng, Y. (2020). From SARS and MERS to COVID-19: A brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. *Respiratory Research, 21*, 224. https://doi.org/10.1186/s12931-020-01479-w