

Survival of SARS-CoV-2 on food surfaces: Discussion

We conducted a laboratory-based study artificially contaminating infectious SARS-CoV-2 virus onto the surfaces of foods and food packaging. We then measured how the amount of infectious virus present on those surfaces declined over time. The foods tested were selected because they are commonly sold loose on supermarkets shelves or uncovered at deli counters or market stalls, they may be difficult to wash, and they are often consumed without any further processing i.e. cooking. The food packaging materials were selected as they are the most used food packaging materials or consumption of the product may involve direct mouth contact with the packaging. They were studied at a range of temperatures and humidity levels and over time periods that reflect their typical storage conditions. SARS-CoV-2 virus was added to the foods and food packaging at a volume that represents respiratory droplets landing on their surfaces. The concentration of virus added was determined by titre of virus we propagated in the method widely used in COVID studies, i.e. using the cell line Vero E6, which expresses the ACE2 receptor for SARS-CoV-2 attachment and entry. Infectious virus was recovered from the foods by the method that gave the highest recovery titre of the three methods tested. These methods were pulsification, vortexing with beads or swabbing. For food packaging, we used vortexing with beads for virus recovery (Warnes et al., 2015). Recovered infectious virus was quantified by plaque assay infection of Vero E6 cells and statistical significance of virus survival between the different incubation parameters and food or packaging materials determined using two-way ANOVA followed by Tukey's multiple comparisons post-hoc tests. For all tests, the LOD was 25 PFU/sample. Therefore, the virus may have survived at low levels, fewer than 25 PFU, throughout the duration of the 7-day tests under the conditions tested. We cannot determine whether this low level of contamination could have an effect on virus transmission and whether this would have any potential health risks.

Results showed that virus survival varied depending on the foods and food packaging examined. For most foods tested there was a significant drop in levels of virus contamination over the first 24 hours. In several cases, e.g., peppers, bread crust, ham, and cheese, infectious virus was detected for several days under some conditions. Even on the surfaces of croissants and pain au chocolate, infectious virus could be found for several hours. For a highly infectious agent such as SARS-CoV-2, which can be transmitted through touching contaminated surfaces and then the face, these findings are highly noteworthy.

7.1 SARS-CoV-2 survival on food surfaces

7.1.1 Fresh vegetables

There have been few studies on survival of *Coronaviridae* on fresh vegetables. On iceberg lettuce the virus could not be detected after 4 days at 4°C (Yépez-Gómez, 2013). In one study (Dhawal et al., 2021), mushrooms demonstrated a reduction in infectious viral titres for SARS-CoV-2 over 24 hours. The authors suggest that this antiviral activity may be due in part to ganodermediol, a sterol in mushrooms, which has been shown to be effective against other viruses. In the same 24 hour-study, levels of SARS-CoV-2 were relatively stable on spinach and lettuce. The virus is expected to survive better at chill temperatures on foods and packaging materials compared to ambient temperatures (Anelich et al., 2020) but for fresh vegetables

presented in this report the difference between survival at chill and ambient conditions, is not so clear cut. For example, this study's results suggests that on the surface of broccoli, SARS-CoV-2 survives for the longest time, up to 5 days, at the ambient temperature of 23°C and 31% RH. On pepper the virus survives for longest (up to 7 days) at the chill temperature of 6°C and 40% RH. The differences are however not statistically significant.

7.1.2 Fresh fruit

There have been few studies on the survival of *Coronaviridae* on fresh fruit. HuCoV-229E has been used as a safer surrogate for SARS-CoV, and latterly SARS-CoV-2, and when inoculated onto strawberries could not be detected during the initial recovery determination tests (Yépez-Gómez, 2013). Apple skin can partially inactivate SARS-CoV-2 within 60 minutes of contact, suggesting that there are chemicals in apple that have antiviral properties. Apple skin contains phenolic derivatives such as catechin, procyanidin, caffeic acid and chlorogenic acid, among other components, which are also found in the pulp and have strong antibacterial activity (Alberto et al., 2006) and anti-viral activity (Blondin-Brosseau et al., 2021). Moreover, the skin contains flavonoids, not present in pulp, such as quercetin glycosides and cyanidin glycosides. Of note, quercetin (3,3',4',5,7-pentahydroxyflavone) at approximately 4.4mg/100g, is a well-known flavonoid with antioxidant properties; its antiviral properties have been investigated in numerous studies, including inhibiting polymerases, proteases, reverse transcriptase, suppressing DNA gyrase, and binding viral capsid proteins (Colunga Biancatelli et al., 2020). SARS-CoV 3CL protease is an important enzyme associated with viral transcription and replication to ultimately aid in viral infection through involvement in the maturation of viral particles and cleavage of the viral capsid. The ability to inhibit the activity of this viral protease could restrict viral replication. Quercetin inhibits SARS-CoV 3CL protease by binding to its GLN189 site, similarly, expressed by SARS-CoV-2 and this provides a direct mechanistic rationale for its experimental clinical use to treat COVID-19. There is an ongoing randomized control trial in Turkey examining the role of quercetin in COVID-19 treatment (Onal, 2020). In the trial, 95 patients with COVID-19 are receiving a 1,000-mg active treatment dose and 113 healthcare workers are receiving a 500-mg dose as prophylaxis. At the time of writing no trial data have been released. A recent study using a related coronavirus, HuCoV-229E, artificially inoculated onto apple skin, demonstrated that viral infectivity declines within a few hours post-contamination on apples, and no infectious virus was detected at 24 h post-contamination (Blondin-Brosseau et al., 2021). The authors demonstrated a similar low recovery efficiency, of just 5.81%, in similar conditions as tested here (21°C and between 30-40% RH), in line with the recovery efficiency in this report of 4.6% from apple skin. The results suggest that the acidic pH of apple skin (approximately pH 3) may influence the infectivity of this related coronavirus, HCoV-229E, by interfering with the virus's spike protein. It is also possible that any protective or cleaning coating added to the apples during factory processing may be toxic to SARS-CoV-2. These coatings may include a thin layer of protective natural wax to prevent dehydration (Schwarcz, 2017). Further investigation is needed to determine which factors are important for inactivation of SARS-CoV-2 on apple skin.

The SARS-CoV-2 virus is expected to survive better at chill temperatures on foods compared to ambient temperatures (Anelich et al., 2020). Although direct comparisons between studies are difficult due to differences in processing methods, the study described here suggests this may be the case for raspberries. There is variable survival of SARS-CoV-2 of at least some, albeit small levels of virus, on raspberry. This might be due to the irregular surface topography of raspberries, making recovery inconsistent. The pitted surfaces of raspberries may protect the virus from desiccation. Flavonoids are also found in raspberries but may not have as great an anti-viral effect as those found in apples, as their levels are much lower (approximately 1 mg/100g) (Määttä-Riihinen et al., 2004).

7.1.3 Baked products and pastries

Several studies have investigated the survival of SARS-CoV-2 under a number of different environmental conditions and have shown that viral persistence under indoor conditions is complex and may be driven by many factors, including surface type, surface porosity, droplet or aerosol size, temperature, relative humidity and matrix (Aboubakr et al., 2020). Direct comparisons between studies are also difficult due to differences in processing methods. One study found that survival on some surfaces was better in low humidity conditions of 20% RH (Biryukov et al., 2020), matching the observation of longer survival at low humidity levels, observed in our study for white bread crusts and brown bread crusts.

Although not statistically significant, it is interesting that under some conditions, virus inactivation was slower over time on white bread crusts than on brown bread crusts. We speculate that possible explanations could be the presence of inhibitory substances, such as arabinoxylan, present in the higher levels of fibre found in brown bread. However, white bread has extra processing compared to brown bread, with the addition of bleaching agents which could potentially be inhibitory as well. It would be interesting to investigate the effects of the separate ingredients on the inactivation of SARS-CoV-2, although this is beyond the scope of this study.

We do not know why the pastries, croissants and pain au chocolat, inactivate the virus so quickly, but we can speculate. The pastries are both coated with a liquid egg wash (Retailer, personal communication), which may have an inhibitory effect on the virus. Eggs have one of the highest levels of arachidonic acid in the human diet. It has been suggested that arachidonic acid and other unsaturated fatty acids which are present in high levels in eggs, may serve as anti-viral compounds (Das, 2020). These anti-viral compounds were suggested to be active against enveloped viruses and the paper suggests they might be active against SARS-CoV-2. Indeed, SARS-CoV-2 was unable to propagate in embryonated chicken eggs, unlike influenza virus (Barr et al., 2020).

7.1.4 Delicatessen items

There have been other studies looking at SARS-CoV-2 survival on deli items, but as far as we know, this study is the first to investigate the virus's survival under defined relative humidity conditions on sliced ham and cheddar cheese. In one study, it was found that SARS-CoV-2 can survive on processed meats for up to 21 days kept in refrigerated conditions (Jia et al., 2022). The authors suggest that these types of deli items have a high saturated fat, protein and moisture content which could prolong potential infectivity of SARS-CoV-2, probably by preventing their desiccation and inactivation. Another research group found similar results, although they only looked at virus survival times up to 24 hours at 4°C on chicken thigh, salmon and prawns (Dhakal et al., 2021). As in this study, they also found that deli items high in protein and saturated fat, with a relatively high-water content, supported longer virus survival. The mechanism of how SARS-CoV-2 and other enveloped viruses attach to foods has yet to be determined. However, these studies and the findings in this report, showing the long survival time of SARS-CoV-2 on sliced ham and cheddar cheese, with their high protein, saturated fat and water content, highlight the importance of proper food handling to prevent any contamination by virus prior to consumption.

The recovery of virus from olives was very low, even after contact of just one minute. Since we used intact whole olives, it is likely that any inactivation of SARS-CoV-2 is due to chemicals on the skin. Although there has been little published work on the anti-SARS-CoV-2 properties of olive skin, olives are a rich source of bioactive polyphenols which have been reported to exhibit antiviral activity against various viruses (Yamada et al., 2009). OliveNet™ is an active directory of phytochemicals obtained from different parts of the olive tree (Bonvino et al., 2018). The research by OliveNet™ identified polyphenols, such as olive secoiridoids as inhibitors of SARS-CoV-2 entry and replication. Olives are also composed of other polyphenols-like flavonoids (i.e., quercetins, also found in apples), triterpenes, and lignans all of which have potential anti-viral effects (Hashmi et al., 2015). Hydroxytyrosol is one of the main phenolic compounds in olives and

its postulated biological activities are antioxidant, anti-inflammatory, anticancer, antimicrobial and antiviral. Hydroxytyrosol's antiviral activity has been reported against influenza-A virus and human immunodeficiency virus (HIV) (Bedoya et al., 2016; Takeda et al., 2021). Although no direct evidence has been shown, these antiviral effects might indicate that hydroxytyrosol is effective against the viral envelope, making SARS-CoV-2 more fragile and hence inactivated in its presence. Several models have suggested that triterpenes, including those found in low levels on olive skin, also have promising structural motifs as SARS-CoV-2 protease inhibitors (Alhadrami et al., 2021).

This study showed that SARS-CoV-2 could survive up to 4 days in brine. A study on enveloped viruses, similar to SARS-CoV-2, found that increased salinity had a detrimental effect on virus stability, reporting lower survival of human and swine influenza viruses as NaCl (sodium chloride or salt) concentrations increased (Poulson et al., 2016). However, another study observed two H5N1 avian influenza viruses: one (A/whooper swan/Mongolia/244/2005) was most stable in water with no added salts (<100 ppm saline) while the other (A/duck meat/Anyang/2001) persisted longest at 15,000 ppm (Brown et al., 2007). The mechanism behind these varying responses to salinity is unknown, but this difference might be due to the host from which the lipid bilayer of the virus was derived and the glycosylation moieties of the surface proteins (Poulson et al. 2016). It appears that the antiviral effect of sodium chloride is strongly dependant on the salt concentration, pH value and on the type of virus.

7.2 Time course of SARS-CoV-2 survival on food packaging materials

7.2.1 PET1 bottles and trays

Potential fomite transmission of SARS-CoV-2 has been studied widely in the last year. This report confirms the findings of previous studies on other enveloped viruses (Warnes et al., 2015; Casanova et al., 2010).

Other studies have used different methods of testing, with many studies looking at RNA recovery rather than viable virus recovery. These different methods make it hard to compare experimental conditions, most previous authors observed long survival of infectious viral particles on plastic and other inanimate surfaces, ranging from hours to weeks. One study showed that SARS-CoV-2 inoculated on glass showed a 2-log₁₀ reduction of culturable virus after 14 days at 4°C (Chin et al., 2020). In this study, on PET1 bottles and PET1 trays, it is unclear why the virus survives for longer at 21°C, 53% RH, compared to 20% and 80% RH. Several studies have investigated the survival of SARS-CoV-2 under several different environmental conditions and have shown that viral persistence under indoor conditions is complex and may be driven by many factors, including surface type, surface porosity, virus in droplets or aerosols, temperature, relative humidity and matrix (Aboubakr et al., 2020). Different inoculum concentrations and volumes, ambient temperatures and relative humidity cause discrepancies and make comparisons difficult. However, most studies broadly agree with our findings, that SARS-CoV-2 can survive on plastic for between 3 days and 7 days, at either 21-23°C or at 4°C (van Doremalen et al., 2020; Liu et al., 2021; Gidari et al., 2021 and Chin et al., 2020). The study by van Doremalen, (2020), was important in providing the first evidence that SARS-CoV-2 could survive for many days on inanimate surfaces (van Doremalen et al., 2020). We have also previously shown survival of the similar HuCoV-229E on plastics for 4-5 days (Warnes et al., 2015). Another study (Casanova et al., 2010) using surrogate viruses, found that virus survival was enhanced by the lower temperature of 4°C, but the relationship between survival and relative humidity was not so clear. The authors suggest that multiple mechanisms may contribute to viral inactivation on surfaces. If viral capsids accumulate at the air-water interface of a solution, structural damage can occur causing viral inactivation. Desiccation (removal of water) may also be important, thus viral inactivation on surfaces may involve both desiccation and interaction at the air-water interface, with the contribution of each depending on both temperature and relative humidity. It is hard to dissect which of the mechanisms is more important in this study.

7.2.2 Aluminium cans

As far as we can ascertain, there have been no other studies on survival of SARS-CoV-2 on coated aluminium cans. There was little difference in virus inactivation in the presence or absence of mucin, and only small differences between different temperatures or relative humidity levels. There have, however, been a few studies investigating the survival of human coronavirus (HCoV) strains on aluminium metal (Kampf et al., 2020; Sizun et al., 2000). Both showed that there was rapid loss of infectivity on aluminium for different HCoV strains and survival was less than 12 hours on aluminium at room temperature. In their review, Kampf found that HCoV-229E and HCoV-OC43 survived for less than 8 hours on aluminium at 21°C; while Sizun showed that very little virus remained after 3 hours on aluminium for HCoV-OC43 and 12 hours for HCoV-229E at 21°C.

However, aluminium drinks cans are complex products; the cans are not pure aluminium. Aluminium cans are made from over 98% aluminium alloys, consisting of 95% aluminium and smaller amounts of manganese, magnesium, chrome, iron, silicon and copper. Aluminium cans are generally then coated with an organic layer to protect the integrity of the cans from effects of the highly acidic foods and drinks and protect against corrosion of the metal leading to leakage of the can and spoilage of the food (Geueke et al., 2016). Since the 1950s, epoxy-based resins are the most used class of aluminium can coatings and in 2013 their market share was estimated to be >95%, including the aluminium cans tested in this study (Anon, 2022a). Epoxy coatings protect the metal from corrosion, can withstand a wide range of food and drinks, and resist heat and acidic conditions. They adhere well to the aluminium surfaces and exhibit sufficient flexibility during most production processes. The most common epoxy coatings are synthesized from bisphenol A (BPA) and epichlorohydrin forming bisphenol A-diglycidyl ether epoxy resins. Most aluminium cans are coated both internally and externally with very thin films, of between 1 and 10 µm thickness. The thin epoxy lacquer coating is then covered with a layer of white base coat paint to provide a stable surface for the various printing inks that are applied, before finally being covered in a varnish lacquer (MPDA 2022). Therefore, the drinks can surface in this study is quite complex and is not pure aluminium. As of yet, there are no other studies to look at the survival of SARS-CoV-2 or any other viruses on epoxy resin varnish.

7.2.3 Composite drinks cartons

As far as we can ascertain, there have been no other studies on survival of SARS-CoV-2 on composite drinks cartons. The virus can persist in an infectious state on composite drinks cartons for several days under certain storage conditions. In some conditions, namely chilled, higher humidity conditions (6°C, 53% and 80% RH) contamination of composite drinks cartons can sustain infectious virus for a significant length of time (up to 4 days). This and other similar findings may warrant decontamination protocols for composite drinks cartons to mitigate further contamination of surfaces and hands.

Composite drinks cartons are complex products. As with PET1 bottles and aluminium cans, the risk of virus transmission comes from drinking straight from the carton as well as touching the carton and then putting their hands to their face. The manufacturer of the fruit juice drinks cartons used in this study produces more than 15 billion composite drinks cartons every year and is the world's third largest supplier of packaging for beverages (Anon, 2022b). A typical composite drinks carton is 75% paperboard, 20% polyethylene and 5% aluminium. The main component, paperboard, provides stability and strength. On the internal surface of the paperboard, a thin barrier layer of aluminium protects against oxygen and light to maintain the nutritional value and flavours of the food in the package. Between the paperboard and aluminium foil is polyethylene enabling the paperboard to stick to the aluminium foil. There are also several layers of polyethylene to prevent moisture getting in or out to keep the products inside fresh, as well polyethylene coated paper for printing on the outer surface. Therefore, the drinks carton surface

onto which the SARS-CoV-2 is artificially contaminated, in this study is quite complex.

7.2.4 Effect of mucin

There were variations on virus inactivation in the presence or absence of mucin. It is unclear from the literature, whether SARS-CoV-2 survives for longer on surfaces in the presence of mucin, with only a few studies being performed. Mixing of highly concentrated inocula with respiratory mucus and saliva increased the infectiousness of influenza virus, allowing its transmission for up to 17 days, confirming the protective role of mucus and saliva for the survival of respiratory viruses (Szpiro et al., 2020). An older study, however, showed that human rhinovirus type 14 suspended in tryptose phosphate broth could survive for more than 20 hours incubation, but for less time when suspended in bovine mucin or in nasal secretions (Sattar et al., 1987). In one study, SARS-CoV-2 virus was diluted in a defined organic matrix, consisting of bovine serum albumin (BSA), mucin and tryptone, following the International Standard ASTM E2197 (ASTM 2018), designed to mimic the composition of body secretions (Riddell et al., 2020). However, this defined organic matrix is not a close compositional match to saliva or respiratory secretions. Other studies have also looked at the inoculation of virus in droplets in a clinically relevant matrix, consisting of an artificial saliva/mucus mixture, and have shown that coronavirus stability is consistently reduced when dry surfaces are inoculated with clinically relevant matrices (Bueckert et al., 2020). Saliva or secretions often contain interfering proteins, which may decrease virus stability in a real-world situation. It was beyond the scope of this study to look at the survival of SARS-CoV-2 in mucus and saliva. It will be important in any future studies to investigate more complex matrices, such as properly defined saliva, nasal secretions, as well as respiratory sputum.

The time for inactivation of SARS-CoV-2 on food packaging was variable, depending on the packaging investigated. The literature suggests that survival of SARS-CoV-2 in the environment is dependent on many factors and often dependent on the experimental design (Abraham et al., 2020). Possible variations include: the virus strains used; culture conditions used; inoculum matrix; presence or absence of additional proteins; volume of virus added; titre of virus used; temperature and relative humidity used; length of time for incubation; porosity of the surface being tested; efficiency of recovery methods used; dehydration of the inoculated virus and exposure to sunlight (Baker and Gibson, 2022). These results highlight the complexity and variability within studies across different laboratories and make comparisons with our study's results difficult.

7.3 General conclusions

In both chilled and ambient conditions at a range of relative humidity levels, some foods and food packaging material can sustain infectious virus for a significant length of time. It should be noted that foods and packaging considered as part of this study were artificially inoculated with SARS-CoV-2 and therefore are not a reflection of contamination levels found on these foods at retail, and lower levels of contamination will require less time to decline to undetectable levels. Several surveillance studies have been performed for the presence or absence of SARS-CoV-2 (Li et al., 2022; Arnaboldi et al., 2022) showing that the proportion of foods or food packaging with surface SARS-CoV-2 contamination is extremely low, but not negligible. Actual virus levels on these foods and food packaging were not measured and virus levels in retail environments have not yet been monitored, as far as we can ascertain.

High saturated fat and high protein foods, such as sliced ham and cheddar cheese, seem to support longer SARS-CoV-2 virus survival, with infectious virus surviving for at least 7 days. By contrast, some foods, such as apples and olives, contain naturally occurring bioactive chemicals which may exhibit potential antiviral properties and therefore contribute to rapid SARS-CoV-2 inactivation. This effect was seen within a few minutes. Some baked products, such as croissants and pain au chocolat, have an egg wash coating, which may have an intermediate antiviral effect. In tests on these pastries, virus survived for a few hours. The results described in this report, on SARS-CoV-2 survival on different food types for short or longer periods of time, reinforce the

need to rigorously follow the guidance on maintaining appropriate hygienic handling measures and display of unpackaged foods.

When considering packaged foods, this study and other similar findings have shown that SARS-CoV-2 may be able to survive, for a prolonged period of time on food packaging. Future studies should recognise that incubating infectious agents on bare aluminium is not the same as on a coated aluminium can, nor on plain cardboard when considering coated drinks cartons.

The public may be interested in the finding that virus may persist in an infectious state, on foods and food packaging surfaces, for several days under certain common conditions. There is the possibility of transmission through contaminated food if the food is in direct contact with the mouth and mucus membranes. The potential implications for public health are unclear since inhalation of respiratory aerosols and droplets is considered to be the main route of SARS-CoV-2 transmission.