








Article

Survival of a SARS-CoV-2 Surrogate on Flow-Pack Polyethylene and Polystyrene Food Trays at Refrigeration and Room Temperature Conditions

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of the current pandemic referred to as coronavirus disease 2019, is spread by direct and indirect transmission between humans, including contact with contaminated surfaces, frozen food, packaging materials, and storage environments. Food contamination may occur in the “farm-to-table” lifecycle through contact with food handlers and environments. In the present study, the survival of a SARS-CoV-2 surrogate (feline coronavirus (FCoV)) at room temperature and refrigeration conditions for different time intervals on two types packaging widely used packaging, namely flow-pack polyethylene and polystyrene food trays, was investigated. FCoV was stable on the flow-pack polyethylene for 48 h and 120 h at room temperature and 4 °C, respectively, while it persisted on polystyrene food trays for 36 h at room temperature and for 120 h at +4 °C. The results of our study highlight the possible implications of food packaging in the spread of SARS-CoV-2 during the current pandemic.

Keywords: SARS CoV-2 surrogate; feline coronavirus; food packaging; flow-pack polyethylene; polystyrene food trays



Citation: Terio, V.; Lorusso, P.; Castrica, M.; Pandiscia, A.; Miraglia, D.; Balzaretti, C.M.; Tantillo, G.; Decaro, N. Survival of a SARS-CoV-2 Surrogate on Flow-Pack Polyethylene and Polystyrene Food Trays at Refrigeration and Room Temperature Conditions. *Appl. Sci.* **2021**, *11*, 3977. <https://doi.org/10.3390/app11093977>

Academic Editor: Andrea Salvo

Received: 17 March 2021

Accepted: 25 April 2021

Published: 27 April 2021

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1. Introduction

Coronaviruses (CoVs) belong to the family *Coronaviridae*, order *Nidovirales*, and their genome is a positive sense single stranded RNA. According to the International Committee on Taxonomy of Viruses (ICTV), the family *Coronaviridae* is classified into two subfamilies, *Letovirinae* and *Orthocoronavirinae*. This last one includes human and animal coronaviruses, and is classified into four genera—alpha, beta, gamma, and deltacoronaviruses [1]. Most human coronaviruses (HCoVs) are betacoronaviruses, like severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and SARS-CoV-2, among others [2].

The coronavirus disease 2019 (COVID-19) pandemic was first identified in December 2019 in Wuhan City, Hubei Province, China, and was then identified as a public health emergency of international concern on 11 March 2020 by the World Health Organization (WHO) [3].

Many studies have shown that the virus is mainly transmitted directly from human to human through the inhalation of airborne droplets (microsized blobs released from human airways when we talk, sneeze, and cough) or virus-laden aerosols [4], but it has been shown that transmission can also occur from SARS-CoV-2 viruses deposited on

an inanimate surface that could infect people through their eyes, nose, and mouth after touching it with their hands [5,6]. Furthermore, it has been shown that high levels of fine particulate matter in the air are related to a greater persistence of SARS-CoV-2 [7]. Therefore, grocery stores and especially supermarkets, are considered a high-risk environments for virus transmission, because of a combination of risk factors, such as enclosed environments; frequently touched surfaces; large numbers of people; and, consequently, difficulty in maintaining physical distance.

Public health authorities, including WHO, Food and Agriculture Organization (FAO) [8], the Centers for Disease Control and Prevention (CDC) [9], and Food and Drug Administration (FDA) [10] in The United States have indicated that there is currently no evidence that the coronavirus that causes COVID-19 can spread through foods or their packaging. Although no direct link has been established yet between COVID-19 infection and food-borne transmission, many recent incidents have highlighted frozen foods as vectors for the long-haul transport of SARS-CoV-2 during the current pandemic. Since July 2020, about nine food contamination incidents were reported, where SARS-CoV-2 was detected on imported foods, mainly packaging materials. Most of these incidents are attributable to frozen shrimp imported from Ecuador, where a novel coronavirus was found on packaging materials, and, in one particular case, SARS-CoV-2 was also detected inside a shipping container [5,11–14].

In early April, the CDC was alerted to COVID-19 cases among workers in several meat and poultry processing facilities [15].

Recently, in the Czech Republic, a local outbreak of SARS-CoV-2 was reported in a grocery stores due to an infected store employee. In this case, all staff and customers wore masks while working and shopping, but eleven customers (including one who even wore gloves in the store and only bought a tub of yogurt) were still infected. This suggests that COVID-19 infection from contaminated food packaging may be rare, but it is possible [16]. The CDC considers COVID-19 infection resulting from handling contaminated food packages to be low-risk, but still recommends cleaning and disinfection [17].

So, viral contamination and foodborne transmission may present a systematic risk in the ongoing pandemic, because food contamination may occur via respiratory droplets, contact, or other routes, during the farming, processing, storage, transport, and retailing process, where foods make contact with different workers and ambient environments in the “farm-to-table” lifecycle.

The aim of this study was to evaluate the survival of coronavirus infectivity at room temperature (RT) and refrigeration conditions for different time intervals on two types of widely used packaging—flow-pack polyethylene (FPP) and polystyrene food trays (PFT). Feline coronavirus (FCoV) belongs to the species Alphacoronavirus 1, and was used as a surrogate for SARS-CoV-2 [18,19]. Alphacoronavirus 1 has previously been employed as a surrogate for SARS-CoV [20,21], and has been shown to have similar persistence on different surfaces [22,23]. Moreover, FCoV manipulation does not require biosafety level 3 (BLS3) facilities.

2. Materials and Methods

2.1. Cells and Virus

Crandell-Rees Feline Kidney (CRFK) cells were used during the experiments. CRFK cells were cultured at 37 °C in a 5% CO₂ atmosphere in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). FCoV strain 25/92 [24] was cultured in CRFK cells to obtain a virus stock, and was titrated in cells through the end-point dilution method. The virus with a titre of 10^{6.50} Tissue Culture Infectious Doses (TCID₅₀/50 µL) was stored at −80 °C and used for the experiments.

2.2. Viral Stability on Different Surfaces Assay

Flow-pack polyethylene and polystyrene food trays materials, each 1 cm² in size, were treated with UVC light (with a wavelength between 200 and 280 nm) for 2 h to obtain

a germicidal effect. Then, they were inoculated with 50 μL of virus ($6.50 \log_{10} \text{TCID}_{50}$), held in an incubator (Forma direct Heat CO_2 incubator, Thermo Fisher Scientific) at room temperature (25°C) with relative humidity (RH 65%) controlled, as well as in a fridge (4°C) with relative humidity (RH 95%) for different time intervals (3, 6, 12, 24, 36, 48, 72, 96, and 120 h). The different hours established for the experiment were chosen considering the average storage time of refrigerated and non-refrigerated foods. In order to mimic airborne aerosols, the viral inoculum was evenly distributed over the entire surface through ten droplets of $5 \mu\text{L}$ each.

After, the materials put in contact with the virus were retrieved at each desired time-point, they were immediately soaked with $450 \mu\text{L}$ DMEM to elute and recover the virus. Washing liquids were collected from each material and were used for the titration tests.

Each sample was titrated onto CRFK cells placed in 96-well microtiter plates. Briefly, ten-fold dilutions of the washing liquids in DMEM were titrated in quadruplicate. The plates were incubated for 72 h at 37°C in 5% CO_2 and the viral titres were determined on the observation of a cytopathic effect (cpe). All of the experiments were performed in triplicate. The limit of detection for the assay was $10^{0.25} \text{TCID}_{50}/\text{mL}$.

3. Statistical Analysis

The virus titres acquired in triplicates are presented as mean \pm SD (standard deviation). Differences in the viral stability on different food packaging materials were tested with student *t*-test (unpaired) in Microsoft Excel 2016. The results were considered significant at $p < 0.05$.

4. Results

We measured virus infectivity sequentially on flow-pack polyethylene (FPP) and polystyrene food trays (PFT) at different temperatures (RT and 4°C) and times (3, 6, 12, 24, 36, 48, 72, 96, and 120 h) used for food packaging.

Different patterns of virus viability were observed according to the packaging type and incubation temperature. In both groups and for both temperatures, the significant reduction vs. the starting inoculum was recorded from the third hour ($p < 0.05$).

At room temperature, a $6.42 \log_{10}$ decrease in the FCoV titre after 48 h was observed on FPP, and the virus viability on PFT showed a $4.92 \log_{10}$ reduction over 36 h, with no infectious virus being detected at 48 h (Table 1). Not surprisingly, with a temperature of 4°C , virus stability increased in both packing types, displaying a $2.92 \log_{10}$ drop on FPP and a $5.92 \log_{10}$ drop on PFT after 120 h of incubation (Table 2). Within 24 h of incubation at room temperature, the virus infectivity decreased by $1.92 \log_{10}$ and $2.92 \log_{10}$ on flow-pack polyethylene and polystyrene food trays, respectively (FPP vs. PFT at 24 h: $p < 0.05$; Table 1). After 24 h at 4°C , the drop in viral titre was $1.67 \log_{10}$ and $2.83 \log_{10}$ on the FPP and PFT, respectively (FPP vs. PFT at 24 h: $p < 0.05$; Table 2).

Table 1. Stability over the time of feline coronavirus on flow-pack polyethylene (FPP) and polystyrene food trays (PFT) at room temperature.

Hours of Incubation	* Viral Titres at Room Temperature FPP	* Viral Titres at Room Temperature PFT
3	5.75 ± 0.25 ^a	4.83 ± 0.14 ^b
6	4.83 ± 0.14 ^a	4.50 ± 0.25 ^a
12	4.58 ± 0.14 ^a	4.08 ± 0.14 ^b
24	4.58 ± 0.15 ^a	3.58 ± 0.14 ^b
36	2.08 ± 0.38 ^a	1.58 ± 0.14 ^a
48	0.08 ± 0.14 ^a	0 ^a
72	0	0
96	0	0
120	0	0

* Viral titres are expressed as mean values of 3 replicates ± DS of log₁₀ Tissue Culture Infectious Doses (TCID₅₀). Different lowercase letters, within each analysis times, represent significant differences between groups ($p < 0.05$).

Table 2. Stability over the time of feline coronavirus on FPP and PFT at refrigeration conditions.

Hours of Incubation	* Viral Titres at Refrigeration Condition FPP	* Viral Titres at Refrigeration Condition PFT
3	5.50 ± 0.25 ^a	5.33 ± 0.29 ^a
6	5.17 ± 0.14 ^a	5.00 ± 0.25 ^a
12	4.83 ± 0.14 ^a	4.08 ± 0.38 ^b
24	4.83 ± 0.15 ^a	3.67 ± 0.29 ^b
36	4.67 ± 0.14 ^a	3.25 ± 0.43 ^b
48	4.58 ± 0.14 ^a	2.83 ± 0.14 ^b
72	4.17 ± 0.14 ^a	2.83 ± 0.15 ^b
96	3.67 ± 0.38 ^a	2.25 ± 0.25 ^b
120	3.58 ± 0.14 ^a	0.58 ± 0.38 ^b

* Viral titres are expressed as mean values of three replicates ± DS of log₁₀ TCID₅₀. Different lowercase letters, within each of the analysis times, represent significant differences between groups ($p < 0.05$).

5. Discussion

The authors in this study conducted experiments on two different food contact materials used for food packaging and investigated the stability of SARS-CoV-2 on flow pack polyethylene (FPP) and polystyrene food trays (PFT) using the feline coronavirus. Although, to date, there is no definitive evidence for the food-borne transmission of SARS-CoV-2, some recent incidents have shown frozen foods as carriers for the long-range transport of the virus, and viral contamination was detected on imported foods and their packaging materials during the current pandemic. In Xinfadi, the biggest wholesale market in Beijing, SARS-CoV-2 was detected on a cutting board used to handle imported salmon [5]. The resulting genomic sequencing identified a European strain of SARS-CoV-2. Subsequently, between July and August 2020, several COVID-19 clusters in China were repeatedly linked to imported frozen raw food. The presence of the virus was detected on food surfaces (frozen chicken wings from Brazil), food packaging materials, and in food storage environments (shipping container from Ecuador) [5,11–13].

Therefore, it is important consider another aspect in the food chain, in particular “farm-to-table”, for which the lifecycle is kept at a low temperature and thus caters to the

survival of SARS-CoV-2. In particular, food handlers could be a main vehicle of the virus because, especially non-symptomatic carriers, can contaminate foods and food packaging materials by droplets expelled from their breathing, coughing, talking, or sneezing [25,26] in enclosed environments.

In addition, the discovery of new outbreaks of SARS-CoV-2 in slaughterhouses and meat processing plants has become a new emergency on the Covid 19 front, and groups of infected people have been found in several countries, such as Portugal, Germany, Italy, England, and Wales [27,28]. Among the key factors that can explain the spread of the SARS-CoV-2 in slaughterhouses, are the following: the large size of the plants with crowding of staff in close workstations, sometimes without personal protective equipment (PPE), or with PPE not properly being used; noise that forces individuals to speak loudly and thus release multiple droplets; cold, which favors the survival of the virus in an environment; and ventilation systems with internal air recirculation in environments saturated with water vapor that favor the spread and permanence of the virus. Furthermore, if we consider the meat supply chain, the phases most at risk are the cutting of the carcasses and the processing of the meat, which take place at a controlled environmental temperature not exceeding 12 °C, maintained at 3–7 °C for storage. This shows that SARS-CoV-2 food transmission may be possible, and recent events have highlighted its potential transmission and presence on food packaging.

To date, as far as we know, no one has investigated the persistence of SARS-CoV-2 on flow-pack polyethylene and polystyrene food trays used for food packaging. Our study showed that a SARS-CoV-2 surrogate, FCoV, can persist at room temperature for 48 h on FPP and 36 h on PFT, while a prolonged survival time, up to 120 h, was observed at 4 °C on both surfaces, albeit with a certain difference in the reduction of viral infectivity (FPP vs. PFT at 4 °C: $p < 0.001$). Our results are in agreement with previous studies showing the persistence of SARS-CoV-2 on plastic materials from 2 to 6 days [16,29,30] and on polystyrene plastic for 92 h [31].

Therefore, our study highlights the possibility that food packaging can be implicated in the spread of SARS-CoV-2 in the current pandemic, especially if low temperatures are used during storage. Future studies should assess the persistence of the virus to temperature changes that may occur during transport from grocery stores or supermarkets to homes, as well as the use of active packaging for viral inactivation. In addition, regarding the release of viral particles into the environment, it is necessary better understand if the decay in infectivity is due to its inactivation, less release in relation to the material characteristics, or a combination of both.

6. Conclusions

COVID-19 infection mainly spreads through common direct transmission, but SARS-CoV-2 was also found on food and, in particular, on food packaging materials. Nevertheless, to date, there is no definitive evidence of SARS-CoV-2 foodborne transmission.

As our results showed that FCoV survives better at refrigeration conditions, special attention should be paid to food packaging contamination in the cold chain of the food industry. For this reason, the strict application of good hygiene practices and the conscious use of personal protective equipment (PPE), such as masks and gloves, and the improvement of food-safety management systems, including good agricultural practices (GAP), good hygiene practices (GHP), and good manufacturing practices (GMP) should be ensured.

Author Contributions: Conceptualization, V.T.; Data curation, P.L. and A.P.; Investigation, P.L. and A.P.; Project administration, V.T.; Software, P.L.; Supervision, C.M.B., G.T. and N.D.; Validation, M.C. and D.M.; Writing—original draft, V.T.; Writing—review & editing, N.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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