Scientific Commentary on:
Bailey *et al*., 2022 "Persistence of coronavirus surrogates on meat and fish products during long-term storage" and associated reports

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Request. On 20 July 2022, ESR received a request via the NZFSSRC from Kaylene Larking, MIA to provide scientific context on the following Bailey *et al*. 2022 paper, with focus also on content from the associated WebMD report and a paper by Velebit *et al.* 2021:

1. Bailey *et al.* 2022. Persistence of coronavirus surrogates on meat and fish products during long-term storage. Applied and Environmental Microbiology 88 10.1128/aem.00504-22 https://journals.asm.org/doi/10.1128/aem.00504-22

2. Quinlan Houghtaling 2022. Coronaviruses can survive on frozen meat for a month. *Web*MD. https://www.webmd.com/lung/news/20220713/coronaviruses-can-survive-on-frozen-meat-for-a-month

3. Velebit *et al*. 2021. Surface adsorption and survival of SARS-CoV-2 on frozen meat. IOP Conf. Series: Earth and Environmental Science 854 doi:10.1088/1755-1315/854/1/012101 https://iopscience.iop.org/article/10.1088/1755-1315/854/1/012101

The specific request was to "*put this into context for us - commenting on the science and the way it has been presented in this article in the light of what we know about transmission etc. Concerning things in the article for us are "survival and replication in the gut" comment in press release (not heard this before I don't think) and reference in the discussion in the paper itself (https://journals.asm.org/doi/10.1128/aem.00504-22) to the pH of meat affecting adherence to the meat surface (attached).*"

Bailey *et al.* study rationale and approach. Due to the considerable number of outbreaks and the transmission of COVID-19 disease at meat processing plants worldwide, the authors posited that this could provide conditions for the contamination of meat, poultry or seafood during animal slaughter, storage or transport. The study investigated the persistence of infectivity of three different viruses as surrogates for SARS-CoV-2 when inoculated onto either beef, pork, chicken, or salmon which was subsequently stored at 4°C or -20°C.

The study independently inoculated 0.5 x 2 cm portions[1] of the different meat types with the three different viruses; the lipid enveloped RNA bacteriophage Phi 6, or two animal coronaviruses murine hepatitis virus (MHV) and transmissible gastroenteritis virus (TGEV). The methods report that a 100 µl volume of 106 to 108 PFU or MPN IU was inoculated per meat sample, but we presume that this was a mistake and that based on the ability of the study to determine up to a 6 log-reduction of the viruses on product over time, actual inoculated amounts were instead 10^6 to 10^8 PFU or MPN IU. The inoculum was allowed to dry on the meat samples, and concentrations of infectious virus remaining on samples were tested at different time periods for up to 30 days following storage at 4°C and -20°C. The concentration

[1] The third dimension was not provided

of infectious virus remaining on the triplicate food samples was determined by plaque forming assay (Phi6) or MPN using cell culture (MHV and TGEV).

Key findings. Overall, the study found that persistence of infectivity differed depending on the viral surrogate, meat type and storage temperature. Key findings were as follows:

- Infectious virus was isolated after 30 days for all viruses and meat types, and at both refrigeration and frozen temperatures. However, there was a reduction in concentration of infectious virus in all samples.
- Following storage at 4°C for 30 days, the reduction in infectivity ranged from as much as 5.68 log reduction for MHV on chicken to as little as 0.73 log reduction for TGEV on pork.
- At least for Phi6 and TGEV, there was less reduction in infectivity when stored for 30 days on all meat types at -20°C compared with 4°C (although the levels of significance were not provided). The reduction of infectivity following storage at -20°C for 30 days ranged from as much as 6.82 log reduction for MHV on chicken to as little as 0.42 log reduction of Phi6 on pork.

Scientific context of the study findings. The authors correctly concede that a limitation of their study is that they used surrogates for SARS-CoV-2 rather than SARS-CoV-2 itself. They also observed widely varying survival characteristics for the three different types of surrogates. Therefore, it is reasonable to assume that the results may also not accurately represent specific survival times of SARS-CoV-2 on foods, although results might provide a rough indication for how SARS-CoV-2 might behave.

The more general finding from this study was that infectious viruses were still detected following 30 days storage at refrigeration and freezing temperatures, albeit at much lower infectious titres. The persistence of infectivity following freezing is indeed expected, given that this is how viruses are stored in the laboratory. Different studies employ different inoculation methods, inoculation concentrations of virus which might vary by up to 5- or 6-$\log_{10}$ infectious particles, and differing incubation conditions. Studies report viral stability in different ways; for example, reporting survival half-lives, the length of time for which infectious virus was still detected, or log-reduction over time. Enumeration units also differ; for example, most probable number, plaque forming units, or tissue culture infectious dose. Therefore, results between studies are often not directly comparable. None-the-less, the findings from Bailey *et al.* (2022) are at least consistent with data from other studies that report on SARS-CoV-2 stability. Some examples of survival times for SARS-CoV-2 following inoculation onto different food products, particularly meats used in the study, and stored at either refrigeration or freezing temperatures are as follows:

- Infectious SARS-CoV-2 remained detectable on pork chops, pork mince and deli turkey for at least 3 weeks at 4°C; note that longer time periods were not tested [1]. SARS-CoV-2 survived for shorter times on beef steak and mince at 4°C (<2 weeks), but the inoculation concentration was lower than that used by Bailey *et al*. This study did not test salmon or chicken.
- A study that assessed SARS-CoV-2 survival on salmon, beef or pork, detected infectious virus after 9 days following storage at 4°C, and after 20 days following storage at -20°C [2]. Longer incubation times were not tested for either storage temperature.

- Infectious SARS-CoV-2 was detected up to 9 days from artificially inoculated salmon incubated at 4°C; however, much lower concentrations were inoculated than used by Bailey *et al*. [3].
- SARS-CoV-2 remained infectious for at least 8 weeks following inoculation into ice cream and stored at either -20°C and -80°C [4]. Infectivity declined by 1.25 to 2 log over this time, depending on the storage temperature.

The Bailey *et al*. study employed very high inoculum concentrations of infectious virus (assuming that concentrations added were 10^6 to 10^8 PFU or MPN IU; see our earlier comment regarding the apparent inoculum concentration reporting error), as is common for other studies that investigate the length of time that viruses remain infectious on different foods and surfaces. Inoculation with a high concentration is necessary to determine the kinetics of reduction of viral infectivity over time. However, inoculation concentrations and incubation conditions do not represent natural contamination and storage scenarios. In general, the length of time that a virus will persist on a food or surface is influenced by the concentration present to start with. Therefore, although viruses persisted for at least 30 days in the study, this might not represent what would occur under natural contamination scenarios.

Robustness of report methodology, analysis and accuracy. In general, we consider that the methodology and reporting of results is sound (although there was a mistake identified with the inoculum concentrations used). However, we identified a number of inaccuracies within the introduction and discussion, with data misrepresented from published studies, incorrect referencing, or referencing sources that did not support the statements of the authors. Identified issues include:

Introduction*:*

- *"Low levels of SARS-CoV-2 have been detected on stainless steel and carboard (sic) held at room temperature with constant humidity [5] and this virus has been recovered at both refrigerated (4°C) and frozen (0°C and −80°C) temperatures on both meat and food contact surfaces [6, 7]".* The comment implies that SARS-CoV-2 was detected on these surfaces in natural scenarios (the previous sentence refers to "sources and exposure and transmission"). Instead, one reference [5] actually artificially inoculated surfaces with SARS-CoV-2 in the laboratory and assessed the length of time for which the virus remained infectious. Therefore, it is expected that the virus would be detected on the surfaces upon which it was inoculated. A second referenced paper [6] was an author's response to an earlier paper [8], neither of which reported on detection of the virus from naturally contaminated environments, or meat and food contact surfaces. The third study [7] is a review.
- *"Other reports, particularly in China, have attributed outbreaks of SARS-CoV-2 to contaminated food*". The wording implies that there is more than one report and more than one outbreak, yet the authors reference a single newspaper article which discusses a single outbreak.
- *"Similarly, Vietnam and New Zealand have also seen outbreaks at 99 and 102 days after the last identified local transmission (15, 16), suggesting another persistent exposure source."* The paper references the New Zealand Ministry of Health website (https://www.health.govt.nz/covid-19-novel-coronavirus/covid-19-data-and-statistics/covid-19-case-demographics#confirmed) which does not provide any context for the comment, as this website is updated regularly but was accessed for the reference over twelve months ago. We presume that the article is referring to the 2020 Auckland August

Cluster, in which the first detected case was an employee at an Auckland Americold cool store, because that outbreak also occurred 102 days since last detected local community transmission. The possibility that imported chilled product or packaging seeded the 2020 Auckland August Cluster outbreak was investigated at the time, and no evidence for this was found. This has been discussed in several reports. First, we discuss this in detail in our NSFSSRC reports [9]. We conclude that "*no evidence was found to support that contaminated imported chilled material packaging was the source of infection for the outbreak. To date, the source of the Auckland August cluster outbreak has not been identified, and the most likely scenario is thought to be border incursion from an infected traveller that acquired the disease while overseas.*" Our conclusions were guided by the Nextstrain narrative by Hadfield et al. (https://nextstrain.org/community/narratives/ESR-NZ/GenomicsNarrativeSARSCoV2/2020-10-01). The outbreak was also reported by Douglas *et al.* [10], where they state that "*Although the index case-patients worked at a cold chain supply facility linked to the border, the source of the outbreak was never established*". Geoghegan *et al*. [11] consider that the "*genomic epidemiologic analysis of the possible origins of the COVID-19 re-emergence in New Zealand in August 2020 was inconclusive, probably because of missing genomic data within the quarantine border facilities and in the global dataset*."

- "*the extent to which SARS-CoV-2 and similar enveloped viruses can survive on foods, such as meat, poultry, and fish, under cold-storage conditions is poorly quantified*". The context was not explained and it is unclear whether this refers to the methodology used in other studies or whether they consider that few studies have quantified this. References were not provided.

Discussion:

- "*Although the World Health Organization (WHO) has stated that transmission of COVID-19 from food or food packaging is unlikely (8, 32, 33), this statement is uncertain and not supported by much available evidence*." Based on our extensive reviews of the literature for the NZFSSRC report [9], we agree with the WHO statement. We identified only limited examples where COVID-19 may have occurred via transmission from food packaging (fomite transmission) and no evidence of foodborne transmission despite (as others have also stated) millions of meals having been consumed since the start of the pandemic.
- "*Therefore, continued efforts are needed to prevent contamination of such foods and their food processing surfaces, worker hands, and food processing utensils such as knives, and there is a need to better address the lack of or inadequate disinfection of these foods prior to meat packaging (34)*." We agree that it is prudent to use good hygiene practices to minimise any possibility of food or food contact surfaces as a source for SARS-CoV-2, but suggesting a need for disinfection of foods before packaging is, in our opinion, not proportionate to the risk.
- "*Only a few studies have evaluated the survival of coronaviruses and their surrogates on meat and fish products or other foods, such as produce*." Surprisingly, although the study discusses studies that looked at survival of other SARS-CoV-2 surrogates on meat or produce, they only considered one where SARS-CoV-2 was used. A treatise of how their results compared with more studies, particularly those that examined SARS-CoV-2 survival on meats and/or at refrigeration and freezing temperatures, would have improved the report. As identified in our NZFSSRC report (Table 2, [9]), there are now a number of studies that report on this. The initial submission of the paper was in March 2022, and accepted in May 2022. It is possible that the manuscript was not updated for recent literature (based on the accession date for the Ministry of Health website).
- A final example where content from published studies has been misrepresented by Bailey *et al.* is discussed later under the heading "**Surface adsorption and survival of SARS-CoV-2 on frozen meat."**

SARS-CoV-2 survival and replication in the gut. According to the WedMD article, "*Researchers said their findings are significant because SARS-CoV-2 can reproduce in the gut, not just in the respiratory tract where most people feel its effects*."

As we discuss in the latest NZFSSRC report, there are a number of studies that show that SARS-CoV-2 can indeed infect intestinal cells [9]. First, these cells express the ACE2 receptor required for SARS-CoV-2 to bind and infect cells [12, 13]. Laboratory studies have shown that SARS-CoV-2 can infect gastrointestinal cell lines [14, 15]. COVID-19 patients can also shed large quantities of SARS-CoV-2 RNA in their faeces [16-24]. Because an infected person can be RT-qPCR-positive for SARS-CoV-2 in faeces for many days or even weeks after they become RT-qPCR-negative in a throat swab, this supports that viral replication in the gastrointestinal tract is occurring [20] (or there is a slow clearance of residual RNA fragments). SARS-CoV-2 is thought to be able to reach the intestinal epithelial cells of the small and large intestine via the blood, not necessarily through the gastrointestinal tract. Further, as we discuss in the report, "*A study whereby Rhesus monkeys were inoculated with an intragastric challenge (via gavage) of very high titres (10^7 PFU in 1 ml buffer) of SARS-CoV-2 resulted in infection of digestive tissues and inflammation in both the lung and digestive tissues [25]. Although sufficient virus remained infectious following transit through the stomach to cause infection, inoculated concentrations were significantly higher than would likely be present in or on food. Furthermore, the inoculum was not mixed with food so the results do not represent a realistic food consumption scenario."*

However, although it is likely that SARS-CoV-2 is capable of replication in the gut, the WedMD article statement does not consider other critical factors which would significantly limit the likelihood of any infectious virus actually reaching the intestines via a foodborne route. First, meat is typically consumed cooked, and proper cooking of the meat will inactivate any infectious SARS-CoV-2 present. Second, if any infectious SARS-CoV-2 is ingested, it is expected to be inactivated by the highly acidic pH of the stomach under most circumstances. As we discuss in the latest NZFSSRC report, "*Coronaviruses are considered to be sensitive to acidic pH and bile [185] and for this reason it is conceivable that a higher infectious dose would be necessary compared with a respiratory route of infection. Researchers have postulated that there may be an increased risk of infection following ingestion of SARS-CoV-2 under conditions that increase stomach pH [186, 187]. This might occur following ingestion of certain foods, for individuals taking medication to reduce gastric activity, or for individuals with low stomach acid (hypochlorhydria) as a result of aging or a medical condition such as atrophic gastritis or Helicobacter pylori infection.*"

Surface adsorption and survival of SARS-CoV-2 on frozen meat. Bailey et al. reports that **"***In a recent study examining SARS-CoV-2 adsorption onto meat surfaces, researchers found that an interplay between pH and electrostatic forces was an important contributor to the survival of the virus (30). In particular when the pH of meat surfaces was 5.5 or less, it was found that viruses firmly adsorbed to the surface of the meat due to a protonated amine group and a hydrogen bond formed with the thin layer of water present on the surface of meat products*."

We reviewed the referenced article by Velebit *et al.* [26]. The article appears to be in the format of a hypothesis presented as a conference proceeding and does not report on actual experimental studies. Based on other cited resources, the article posits that SARS-CoV-2 will adsorb more strongly to meat due to the interplay of electrostatic forces between the virus and the meat if the meat is at a pH of 5.5 (which they state can occur several hours after slaughter). They state that the SARS-CoV-2 particle will become positively charged due to the protonation

of both the carboxylate and amine groups and that the protonated amine groups will electrostatically bind to the electron-rich meat matrix. At the same time, a hydrogen bond will be established between the COOH group of the viral protein and oxygen in hydroxyl groups present on meat surfaces. Assessing the validity of this hypothesis is outside of our area of expertise, and we are unaware if this has actually been tested experimentally.

It is also unclear to us how increased adsorption might influence viral survival on meat during freezing. The authors state in their abstract that "*the strong surface adsorption and ability of SARS-CoV-2 to survive meat freezing indicate a potential risk of virus transmission by meat"*; i.e., the two points are considered separately. In their conclusion they state that "*Once adsorbed, SARS-CoV-2 is capable of surviving chilling and most probably freezing temperatures*." The influence of pH on viral adsorption to meat and SARS-CoV-2 survival in frozen meat are also addressed in separate sections. Under the heading "*SARS-CoV-2 survival in frozen meat",* they reference a review by Han *et al*. [27] that discusses data from a preprint article that has now been withdrawn on SARS-CoV-2 survival on buffer containing meat during freezing and refrigerated storage. They also reference studies that report SARS-CoV-2 survival on food at non-freezing temperatures. However, the influence of adsorption to the meat on survival of the virus during frozen storage, was not clear to us.

Key findings. The key findings of this review are as follows:

- The Bailey *et al.* study found that three different surrogate viruses remained infectious following 30 days storage at refrigeration and freezing temperatures, albeit often at much lower infectious titres. Survival characteristics varied by virus type, food type and temperature, so results might only provide an approximate indication for how SARS-CoV-2 might behave. However, the persistence of infectivity following freezing is indeed expected, given that this is how viruses are stored in the laboratory, and comparable results were found in other studies that examined SARS-CoV-2 stability on food.
- The high inoculation concentrations used in the study do not represent natural contamination and storage scenarios. In general, the length of time that a virus will persist on food or surfaces is influenced by the concentration present to start with. Therefore, although viruses persisted for at least 30 days in the study, this might not represent what would occur under natural contamination scenarios.
- WebMD statement: "*Researchers said their findings are significant because SARS-CoV-2 can reproduce in the gut, not just in the respiratory tract where most people feel its effects*." SARS-CoV-2 is indeed likely to be capable of replication in the gut. However, the statement does not take into account other critical factors which would significantly limit the likelihood of any infectious virus actually reaching the intestines via a foodborne route. The likelihood of infectious virus from contaminated meat reaching the intestine is remote because proper cooking of the meat will inactivate any infectious SARS-CoV-2 present, and if any infectious SARS-CoV-2 is ingested, it is expected to be inactivated by the highly acidic pH of the stomach under most circumstances.
- Bailey *et al.* statement: "*In a recent study examining SARS-CoV-2 adsorption onto meat surfaces, researchers found that an interplay between pH and electrostatic forces was an important contributor to the survival of the virus*". We consider that the authors have overinterpreted the conference proceedings article by Velebit *et al..* The article hypothesised how such an interplay might play out, but did not present experimental data.

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