Seeding of outbreaks of COVID-19 by contaminated fresh and frozen food

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Author Contributions

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AR provided insights into the food supply chain and processing, drafting relevant sections of the manuscript

AK and DE undertook all the microbiology work

AR provided the statistical inputs

All authors made significant contributions to the paper and approved the final version

Abstract

An explanation is required for the re-emergence of COVID-19 outbreaks in regions with apparent local eradication. Recent outbreaks have emerged in Vietnam, New Zealand and parts of China where there had been no cases for some months. Importation of contaminated food and food packaging is a feasible source for such outbreaks and a source of clusters within existing outbreaks. Such events can be prevented if the risk is better appreciated.

Main text

An outbreak of SARS-CoV-2 in the Xinfadi wholesale market in Beijing has been accredited by Chinese authorities to imported contaminated food (1). It occurred 55 days after the last identified locally acquired case in Beijing. Steps to mitigate the risk of similar outbreaks have been taken within China, yet elsewhere this risk is underappreciated. Vietnam and New Zealand have new unexplained outbreaks 99 and 102 days, respectively, since their last identified local transmissions (2,3).

It is possible in these regions that eradication was never truly achieved and that there had been ongoing unidentified transmission. Alternatively, occult transmission chains can be seeded through travelers but this mechanism would require a false negative swab in an asymptomatic individual or failure to ensure quarantine. For occult transmission to lead to recrudescence of an outbreak months since the last detected case would require an ascertainment rate that for most settings is implausibly small, as demonstrated through simulations (supplementary material). Even for a reproduction number of 1, a transmission chain that avoided stochastic extinction, and only one in twenty infections being detected, the chance of reaching 20 generations without detection is at most one in a thousand.

Another possibility to explain reemergence is transport of contaminated products such as foodstuffs. A well recognised feature of the COVID-19 pandemic is the number of clusters within meat and seafood processing facilities. In the UK, outbreaks of COVID-19 caused disruption in a poultry processing plant and in an establishment producing ready meals for supermarkets (4). Fish processing in tuna canneries in Portugal and Ghana was suspended after workers in both countries tested positive for COVID-19 (5,6). Abattoirs in Australia have closed following large clusters amongst its workers (7,8). In Germany, more than 1,500 workers tested positive for COVID-19 at one of the largest slaughterhouses in Gütersloh culminating in a lockdown of two districts and over 600,000 people (9).

The Beijing Xinfadi wholesale food market event has lead to concerns that imported contaminated food could seed new clusters. SARS-CoV-2 was detected on workers and environmental samples, including a cutting board used to slice imported salmon. A swabbing campaign directed at millions of nearby residents as well as all workers at the market and the broader Beijing food chain revealed 335 COVID-19 cases (10).

Chinese authorities acted quickly to suspend import of salmon from Europe (11,12), and later imports from food premises where outbreaks of COVID-19 have occurred among workers, affecting the US, Germany and Brazil among others (13). In July, China also suspended imports of shrimp from three Ecuadorean processing plants after detecting SARS-CoV-2 on shipments (14).

The feasibility of this "non traditional" transmission mechanism is currently debated. At 21–23°C no viable SARS-CoV-2 was found after 4 hours on copper surfaces, 24 hours on cardboard and after 3 days on stainless steel and plastic surfaces (15). We have assessed the survival of SARS-CoV-2 on refrigerated and frozen meat and salmon over 3 weeks to assess the potential of outbreaks being seeded by imported contaminated food.

Vero-E6 cells (ATCC# CRL-1586) were maintained in Dulbecco's modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin. SARS-CoV-2, isolate BetaCoV/Singapore/2/2020 (Accession ID EPI_ISL_406973), was used as virus inoculum. All work with SARS-CoV-2 was performed under BSL3 containment at the Duke-NUS Medical School ABSL3 laboratory.

Individual pieces of salmon, chicken and pork sourced from supermarkets in Singapore were sliced into 500 mm^3 cubes and 200 µl of 3×10^6 $TCID_{50}$/ml SARS-CoV-2 was added to each cube. The samples were stored at 3 different temperatures (4˚C, –20˚C and –80˚C) and harvested at specified time points (1, 2, 5, 7, 14- and 21-days post-inoculation). Following incubation, 150 µl of the virus inoculum was transferred to a new tube and frozen at –80°C until titration. Three replicates were performed for each condition. The cell-free virus titre ($TCID_{50}$/mL) for each sample were determined by limited dilution. The limit of detection (LOD) was 5×10^1 $TCID_{50}$/mL. GraphPad Prism (Version 5.04) was used to perform one-way ANOVA, followed by Dunnett's Multiple Comparison Test.

The titre of SARS-CoV-2 remained constant at 4°C, –20°C and –80°C for the duration of the experiment (Fig 1). Infectivity was maintained for 3 weeks in both the refrigerated (4°C) and frozen (–20°C and –80°C) samples. No significant difference was observed between SARS-CoV-2 recovered after incubation with or without the presence of food.

The WHO advises that it is very unlikely that people can contract COVID-19 from food or food packaging (16). While it can be confidently argued that transmission via contaminated food is not a major infection route, the potential for movement of contaminated items to a region with no COVID-19 and initiate an outbreak is an important hypothesis. It is necessary to understand the risk of an item becoming contaminated and remaining so at the time of export, and of the virus surviving the transport and storage conditions.

The clusters of infection of COVID-19 among workers in slaughterhouses and meat processing facilities in many countries can be attributed to factors that promote transmission of virus directly between workers, such as crowding, poor ventilation, and shouting in close proximity due to high ambient noise levels. Workers may go to work when infected, they may live in crowded housing, and travel on crowded transport. Environmental contamination at the work site is likely to be prolonged due to low temperatures, metal surfaces and lack of UV light (17,18).

With a significant burden of virus present in infected workers and the environment then contamination of meat with SARS-CoV-2 is possible during butchering and processing. The killing lines in abattoirs generally run at ambient temperature but the process later moves into a controlled environmental temperature of not greater than 12°C for the breakdown of carcasses and meat is maintained at 3–7°C as legislated by food regulations. The processing of meat and poultry is generally carried out manually in crowded conditions. Salmon processing is, in contrast, highly automated with filleting and cutting performed by machines, and minimal handling by workers. However, where such processing is carried out manually in crowded conditions the risk of contamination increases.

Our laboratory work has shown that SARS-CoV-2 can survive the time and temperatures associated with transportation and storage conditions associated with international food trade. When adding SARS-CoV-2 to chicken, salmon and pork pieces there was no decline in infectious virus after 21 days at 4°C (standard refrigeration) and –20°C (standard freezing).

Contamination of food is possible, and virus survival during transport and storage is likely. Food transportation and storage occurs in a controlled setting akin to a laboratory. Temperature and relative humidity is consistent and maintained and adverse conditions such as drying out is not permitted for the integrity of the food. In quantifying the viral titre we can reasonably assess a rate of decline in infectivity, which did not occur in any of the conditions we assessed.

We believe it is possible that contaminated imported food can transfer virus to workers as well as the environment. An infected food handler has the potential to become an index case of a new outbreak. The international food market is massive and even a very unlikely event could be expected to occur from time to time.

Efforts to avert the risk of COVID-19 outbreaks seeded by contaminated food must begin at the source; that is food processing premises. These include frequent hand washing, cleaning of food contact surfaces, materials and utensils. Fitness to work protocols should be in place and unwell staff should be excluded. Furthermore, the conditions under which workers in our food chain must be reviewed to ensure that our food is safe. Financial support needs to be given to unwell workers to ensure no disincentives to self-isolation or presenting for a test. PPE usage needs to be overseen and social distancing in and out of the workplace needs to be supported.

In receiving markets at the other end of the supply chain, food cannot be decontaminated, however, added precautions to ensure good hand hygiene and regular cleaning of surfaces and utensils is important. Consumers should wash their hands after touching uncooked products and ensure that food is well cooked.

Our findings, coupled with the reports from China of SARS-CoV-2 being detected on imported frozen chicken and frozen shrimp packaging material, should alert food safety competent authorities and the food industry of a “new normal” environment where this virus is posing a non-traditional food safety risk.

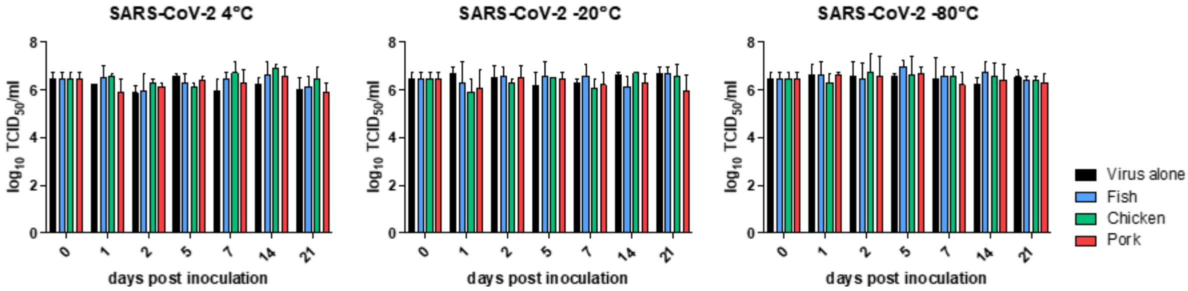

Figure 1: Quantification of infectious virus over 21 days. Viral titres were determined by limiting dilution. Titres are expressed as mean ± SD $\log_{10}$ $TCID_{50}$/mL. SARS-CoV-2 was stored alone or in the presence of fish, chicken or pork and tested under refrigeration (4°C); and frozen (-20°C and -80°C).

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Appendix to Fisher et al

To assess the feasibility of long chains of occult infection leading to clusters some time after a community was thought to have been clear of infection, we built a branching process simulation model. Each case is assumed to generate a Poisson-distributed number of secondary cases with mean ρ. We seed the chain with a single unidentified case in generation 0, the untraced and unobserved daughter infection of a case. The number of infections in generation g of the chain is denoted N_g, which has distribution $Po\left(N_g\rho\right)$. Each case after the initial infection that seeds the chain is ascertained with probability α. The chain is simulated forward for 30 generations or until 1 000 000 cases or until the first case is ascertained. We then calculate the number of generations passed until the chain is uncovered or becomes extinct, and the probability there is on-going occult transmission in each generation. The probability the chain of infection is detected and the expected number of infections are derived through Monte Carlo simulation with 100 000 draws.

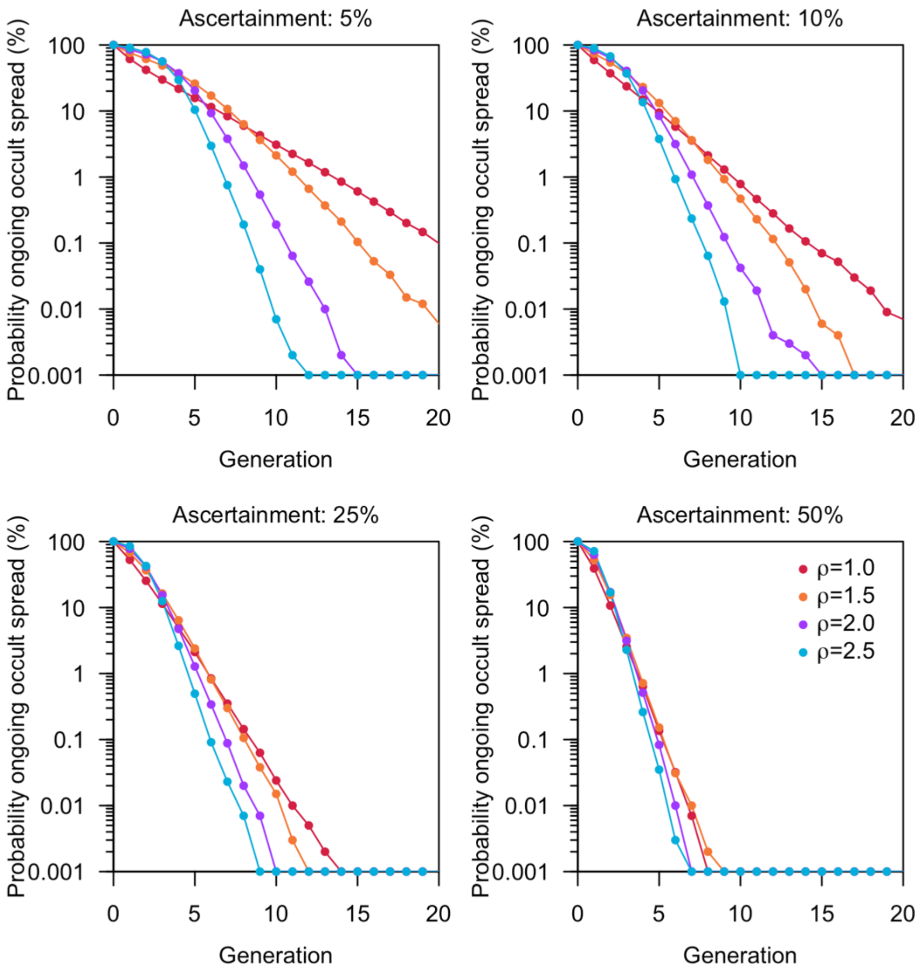

Figure S1: Probability of ongoing undetected transmission for the first 20 generations for four values of the ascertainment rate (α = 5%, 10%, 25% and 50%) and reproduction number (ρ = 1, 1.5, 2, 2.5). Probabilities <1/100 000 are set to 1/100 000 for graphical purposes.

It is very unlikely that ongoing occult transmission would be maintained for more than ten generations except for low reproduction numbers and very low ascertainment rates.