

2021中国国际食品安全与质量控制会议

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Dr Guerrino Macori

Investigating the Presence of SARS-CoV-2 in Selected Foods and Food Production Environments Using Harmonised RT-qPCR and WvGS Protocols

www.ucd.ie/cfs







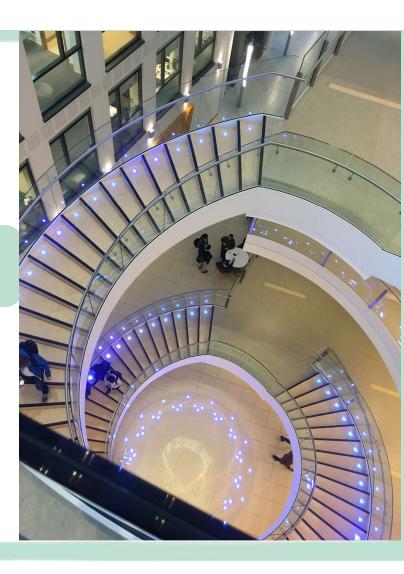






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Application of techniques for sequencing and WGS analysis

Professor Séamus Fanning





🐯 protocols.io

- Standard operating procedures (SOPs)
- bacterial culture and extraction
- library preparation
- bioinformatics workflow
- genomic comparison of 22 isolates
- whole viral genome sequencing (WvGS) of SARS-CoV-2 and computational analysis

Application of techniques for sequencing and WGS analysis

Professor Séamus Fanning

SARSfood

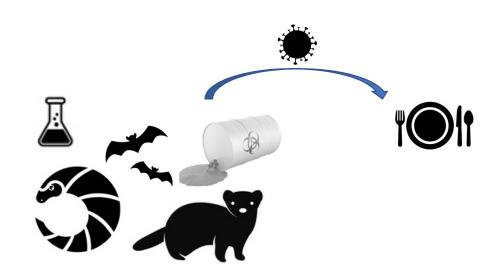
 Investigating the presence of SARS-CoV-2 in selected foods and food production environments using qPCR and WvGS

Prof. Seamus Fanning, Lauren Russell, Francisco Cores Rodriguez, Alexander Floss-Jones, Charlene Bennet, Siobhan McCarthy



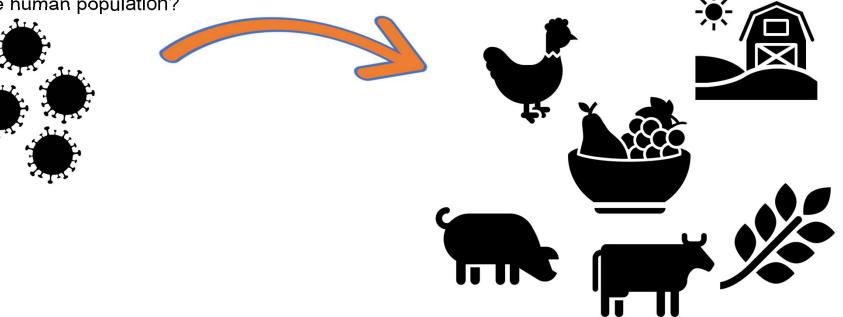


Possible source of virus SARS-CoV-2, animals may be natural hosts. Is it a zoonoses?

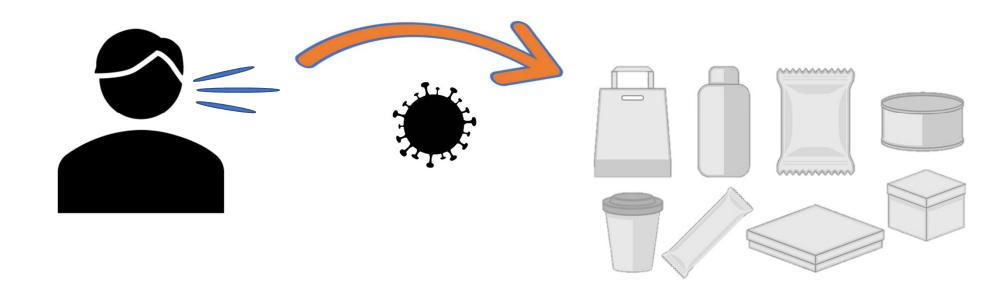


In particular, the role (if any) of domesticated food-producing animals and the meat derived from them

- necessitating the development of suitable diagnostic protocols; implementation of intervention measures along the food chain and recognising transmission routes.
- Could also be possible for SARS- CoV-2 a series of barrier jumps?
- Are food preparations and cooking habits considered a risk factors for the reemergence of this virus in the human population?



Of concern are **processed foods and food packaging**, wherein the SARS-CoV-2 could reach a susceptible consumer following cross-contamination from a previously infected individual (after bouts of coughing or sneezing).



Although there is no direct and credible evidence to state that COVID-19 is a food-borne disease, contact with food and food packaging surfaces remains a low risk in the context of the ongoing pandemic.

The COVID-19 pandemic has presented several challenges to stakeholders along the food chain. These must be overcome in order to ensure food safety and will involve the ability to reliably detect the virus in various food matrices and the built food production environment. As we move towards a post-pandemic era, public health surveillance will involve not only screening human populations, but also monitoring of foods; preparation surfaces and related environments, to assess risk and implement scientifically- based countermeasures.

Although, several **meat processing plants (MPPs)** in the US, Europe and elsewhere, suspended operations, when workers tested positive, though no food recalls were instituted















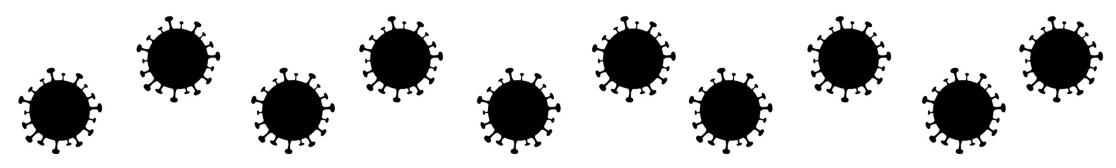




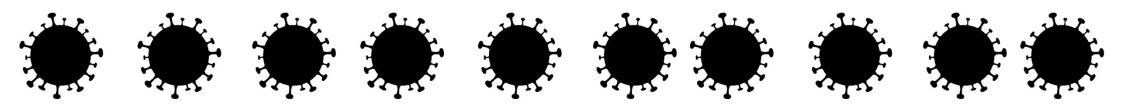


This project would set out to provide with evidence on the following:

- describe the detection of SARS-CoV-2 in selected food matrices (raw meat; selected vegetables and fruits);
- describe the detection of SARS-CoV-2 in selected built food processing environments (FPP) to include sampling of various locations (including taps; door handles; refrigerator handles; smear samples from air conditioning units and other locations considered high-touch);
- determine the whole virus genome sequence (WvGS) of all viral strains recovered from selected food matrices and locations within the built food production environments;
 - using WvGS make provide data describing a comparative genomic study to provide information on the potential relationships between these foods, ecological niches and those sequences deposited within GISAID

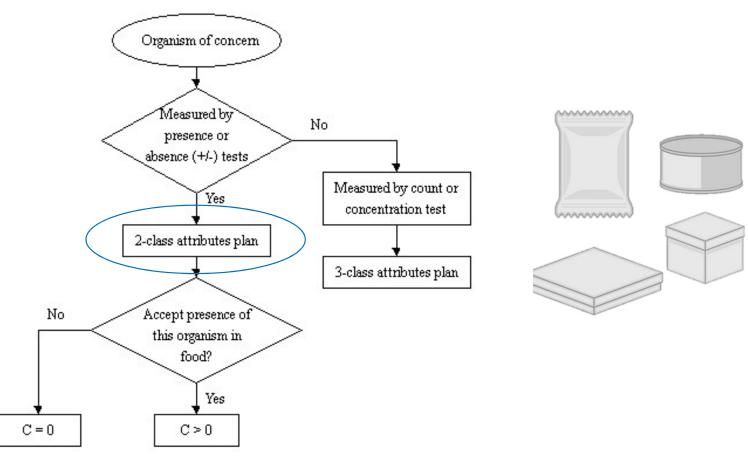


- Identification of SARS-CoV-2 RNA in selected food production environments and foods, by RT-qPCR
- Characterisation of qPCR positive samples by whole virus genome sequencing (WvGS) and comparative genome analysis of these viral strains with those globally available



Sampling plan: the model





Assuming that in this study is preferred a sampling plan with the highest confidence on the results, among the binomial staged sampling plans, is chosen the table with the confidence limit of **0.99 and 0.10** as the upper confidence level.



Sampling plan: the model

Confidence Limit .99		0 out of:	1 out of:	2 out of:
A	.30 ucl*	15	22	27
В	.25 ucl	19	27	34
С	.20 ucl	24	34	43
D	.15 ucl	35	47	59
E	.10 ucl	51	73	90
F	.05 ucl	107	161	190



Using this approach, for each type of food is ideally preferred a **number of 51 samples**.



1. Food samples

- Raw meat
- Vegetables
- Fruit

2. Abiotic surfaces of high-touch locations

- Air conditioning units;
- Taps;
- · Door handles:
- Refrigeration handles
- Others

3. Sewage from selected food/meat processing plants

Sala-Comorera et al., 2021

Journal Pre-proof

Decay of infectious SARS-CoV-2 and surrogates in aquatic environments

Laura Sala-Comorera^a, Liam J. Reynolds^a, Niamh A. Martin^a, John J. O'Sullivan^b, Wim G. Meijera*, Nicola F. Fletcherc.

- a. UCD School of Biomolecular and Biomedical Science, UCD Earth Institute and UCD Conway Institute, University College Dublin, Dublin 4, Ireland
- b. UCD School of Civil Engineering, UCD Dooge Centre for Water Resources Research and UCD Earth Institute, University College Dublin, Dublin 4, Ireland.
- c. UCD School of Veterinary Medicine and UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland
- * Corresponding author: Wim G. Meijer, Tel: (+353) 17162778, Email: wim.meijer@ucd.ie

Graphical abstrac





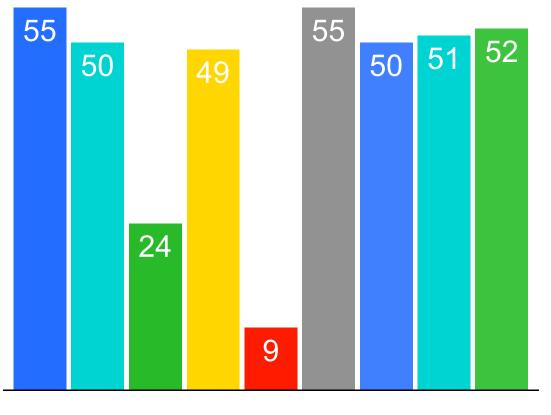




Highlights

. T₉₀ of infectious SARS-CoV-2 at 4°C was 3.8 and 2.2 days in river and seawater

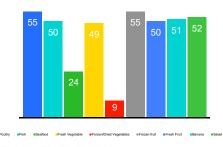




Samples:

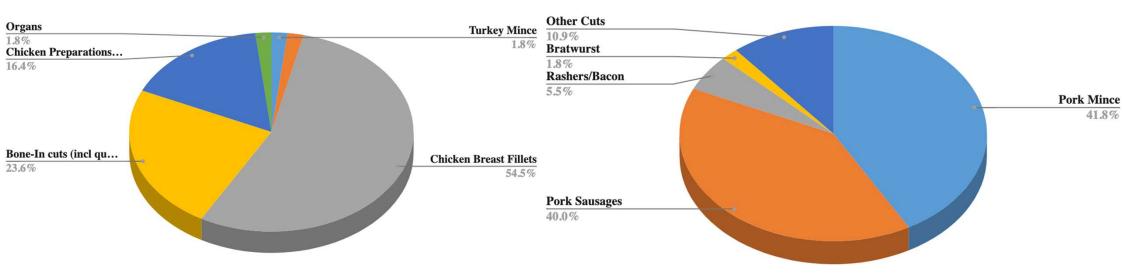
105 raw meat101 fresh vegetables RTE157 fruit RTE (fresh/frozen)

■ Poultry ■ Pork ■ Seafood ■ Fresh Vegetable ■ Forzen/Dried Vegetables ■ Frozen fruit ■ Fresh Fruit ■ Banana ■ Salad

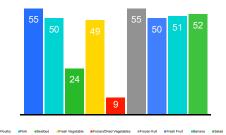


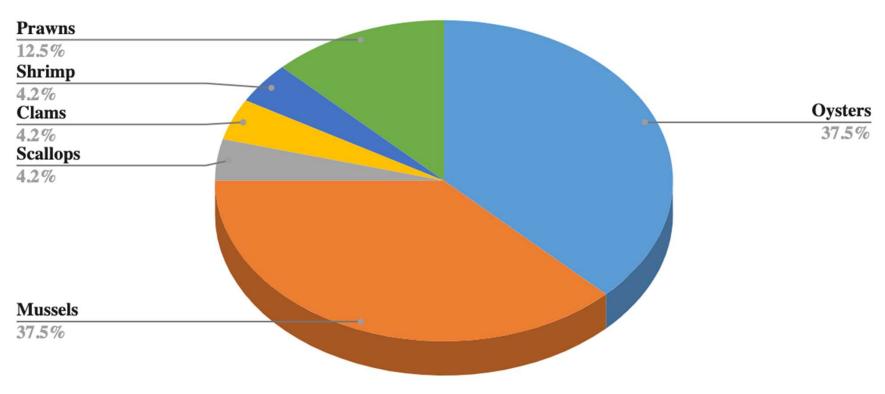
Poultry Sample Types - WHO Food SARS-COV-2

Pork Sample Types - WHO Food SARS-COV-2

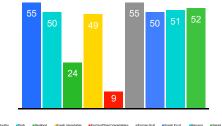


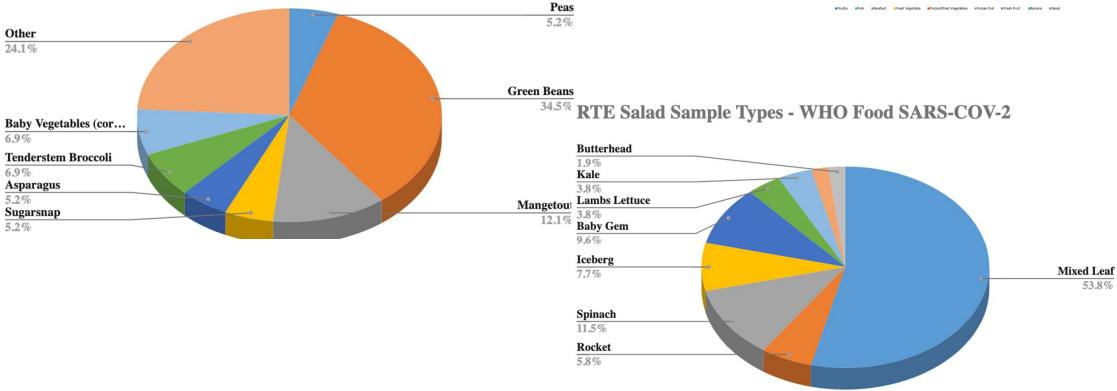
Seafood Sample Types - WHO Food SARS-COV-2



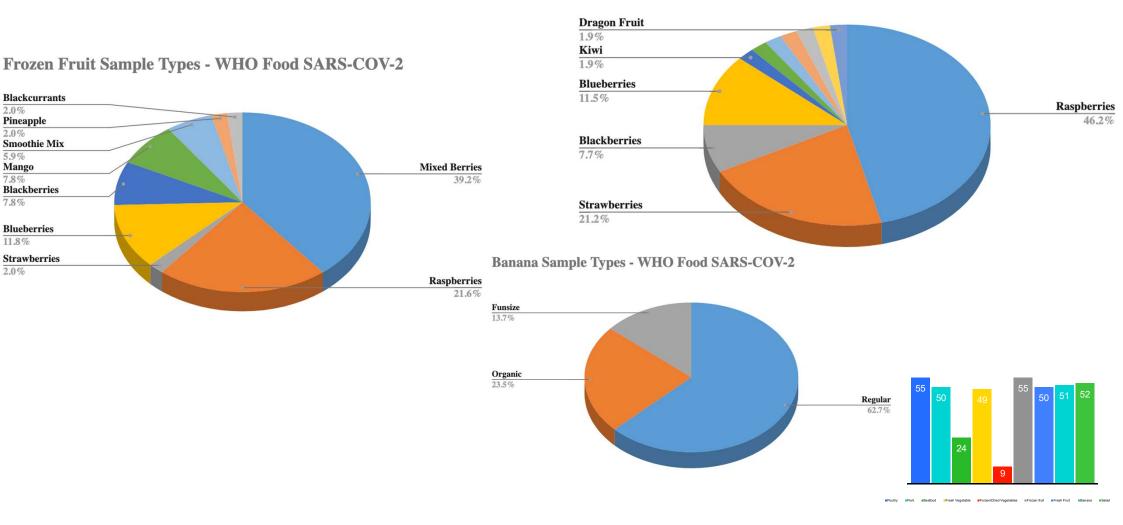


Vegetable Sample Types - WHO Food SARS-COV-2





Fresh Fruit Sample Types - WHO Food SARS-COV-2



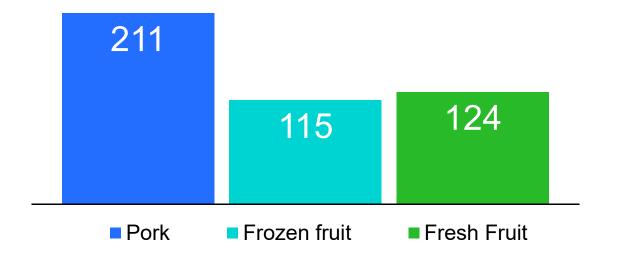


All the packaging were tested as surface swabs, including skin of bananas

367 samples



Retrospective study



Pork meat

• Small sausages: 74

• Large sausages: 63

• Fermented sausages: 45

• Pork liver: 29

Small Fruits

• Raspberry: 61

• Strawberry: 63

• Frozen Raspberry: 56

• Frozen Strawberry: 59

Abiotic surfaces of high-touch locations



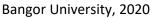




Wastewater samples

The samples were collected from a **line of untreated wastewater** (e.g., lift stations, interceptors, manholes) includes **waste from building use** (e.g., toilets, showers, sinks), which contains human faecal waste, as well as the waste of industrial use/rainwater.



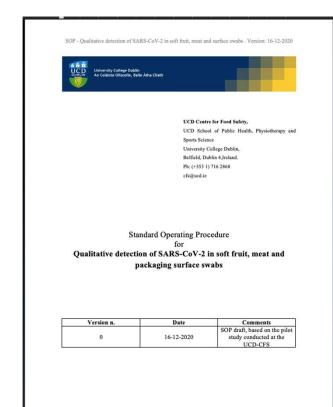




Wastewater samples

Collection of the samples:

- composite samples are collected by pooling multiple grab samples at a specified frequency over a set time period typically 8 hours for wastewater surveillance. You can collect composite samples of untreated wastewater manually (e.g., one sub-sample per 500 cubic meters of flow or 125 ml every 2 hours).
- rapidly in one time (grab sample) reaching the point for the sampling. However, grab samples may be less representative of community fecal contributions than composite samples. For untreated wastewater and sludge, grab samples represent a single moment in time and are highly influenced by daily fluctuations in wastewater flow and composition. If this is the case, we suggest taking the sample at the highest pick of usage in the working day.





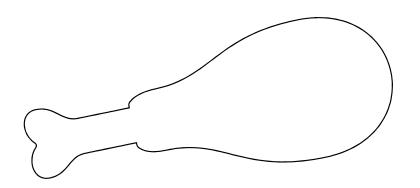
- Remove the individually wrapped swab from the envelope and immerge immediately into sterile tubes containing 1-3 mL of VTM;
- Lightly tap the swab tip to the inside of the transport container to remove excess transport medium (liquid);
- Use swab to sample surface areas of interest (25 cm² is commonly used but a specified area is not required). Swab with moderate pressure while moving in at least two different directions and rotating the swab so that the entire swab surface area is used. Avoid letting the swab dry completely.
- Immediately after sampling, place the swab back into the transport container, cut the stick at the breakpoint, bending gently and screw the cap on tightly.

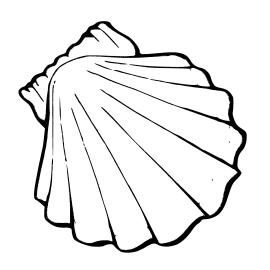


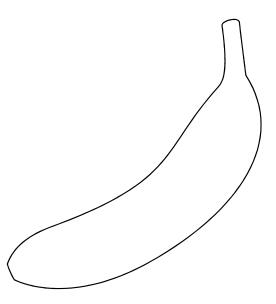
Date	Swab No.	Area	Location	
15-Feb	1	Blank	Blank	
15-Feb	2	Visitors entrance	Door inside	
15-Feb	3	Whey SM office door	Door inside	
15-Feb	4	Dairy Panel room door	Door outside	
15-Feb	5	ED Room Door Handle	Door inside	
15-Feb	6	Whey Lab door	Door inside	
15-Feb	7	Liquid lab door	Door outside	
15-Feb	8	Photocopier ED	Touch panel	
15-Feb	9	Photocopier Admin	Touch panel	
15-Feb	10	Driver fridge	Door outside	
15-Feb	11	Driver freezer	Door outside	
15-Feb	12	Foam Machine	Handle	
15-Feb	13	Whey office air con unit	Unit on ceiling	
15-Feb	14	Whey SM keyboard	Keyboard	
15-Feb	15	Dairy SM office door handle	Inside handle	
15-Feb	16	ED panelroom keybaord	Composite samples	
15-Feb	17	Crystallisation keyboard	Keyboard	
15-Feb	18	Chair Handle in Whey office	Peters chair	
15-Feb	19	Elevator N3	Buttons	
15-Feb	20	Control room phone ED	Composite samples	
15-Feb	21	Casein Keyboard	Composite sample	
15-Feb	22	Casein door handle	Door handle outside	
15-Feb	23	Security hut	Antibiotic touch panel	
15-Feb	24	Canteen	Composit sample of microwave handles	
15-Feb	25	Bag off door handle	Door inside	

75 samples high touch swabs6 wastewater samples

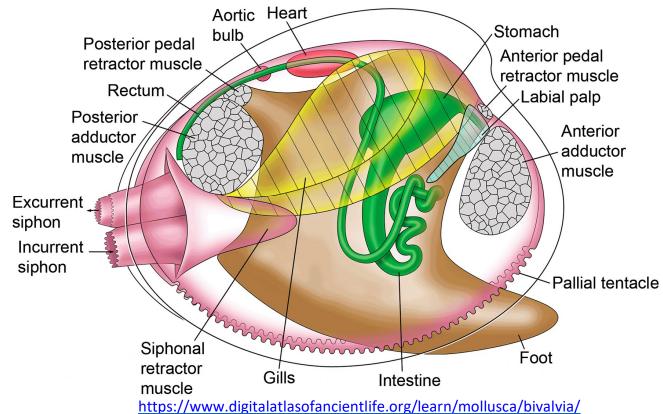
- Meat products
- Bivalve Molluscan Shellfish and Crustacean
- Fruits and vegetables







ISO 15216-1:2017 Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR Meat products - validated SOP - UCD National Virus Reference Laboratory (Charlene Bennet)





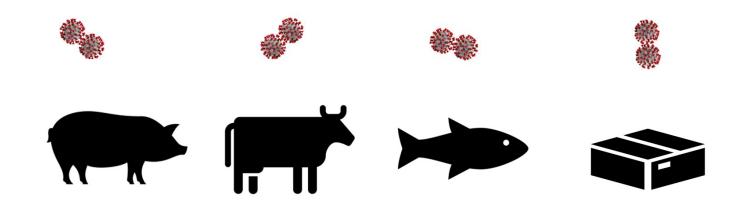








Experimental spiking of packaging, pork and meat (beef and salmon fillet)
Recovery of virus particles, cell cultures (counts)

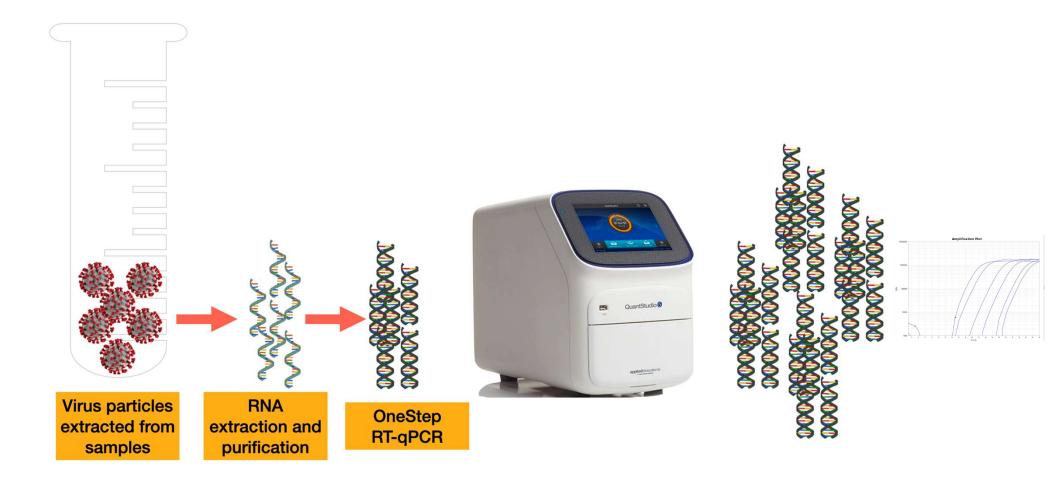


Automated purification of viral RNA using the QIAamp Viral RNA Mini Kit on QIAcube Connect

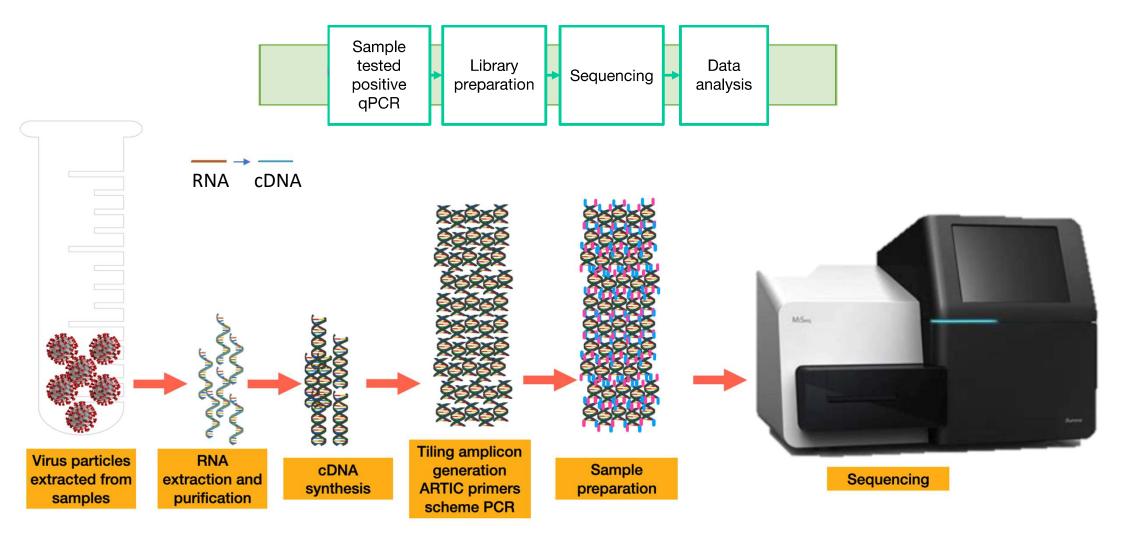




Methods – Reverse Transcriptase qPCR (RT-qPCR)



Methods – tiling amplicon sequencing





Implementation of a high-throughput Illumina MiSeq-based sequencing platform

Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples

Joshua Quick¹, Nathan D Grubaugh² ⊙, Steven T Pullan³, Ingra M Claro⁴, Andrew D Smith¹, Karthik Gangavarapu², Glenn Oliveira⁵, Refugio Robles-Sikisaka², Thomas F Rogers².6, Nathan A Beutler², Dennis R Burton², Lia Laura Lewis-Ximenez', Jaqueline Goes de Jesus⁸, Marta Giovanetti^{8,9}, Sarah C Hill¹⁰, Allison Black^{11,12}©, Trevor Bedford¹¹, Miles W Carroll^{3,13}, Marcio Nunes¹⁴, Luiz Carlos Alcantara Jr. ⁸©, Ester C Sabino⁴, Sally A Baylis¹⁵, Nuno R Faria 10, Matthew Loose 16, Jared T Simpson 17, Oliver G Pybus 10, Kristian G Andersen 2.5 & Nicholas J Loman 1

**Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK. "The Scripps Research Institute, La Jolla, California, USA.
**Public Health England, National Infections Service, Proton Down, Sulborry, UK. "Department of Infectious Disease and Institute of Project Medicate, University
**Public Health England, National Annual Scripe Institute, Land Science Institute, La Machadiema, USA. Annual Annual Scripe Institute, Land Scripe Instit

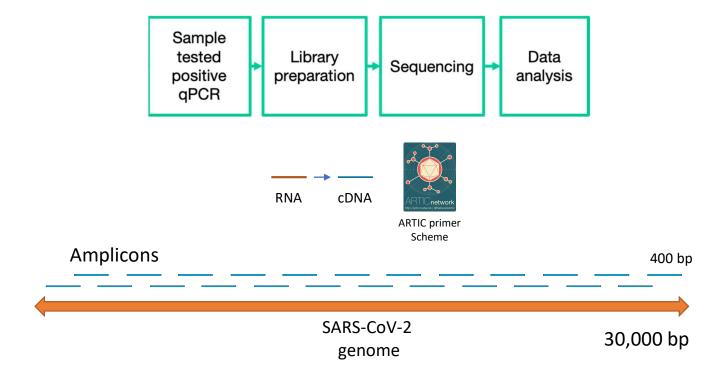
Genome sequencing has become a powerful tool for studying emerging infectious diseases; however, genome sequencing directly from clinical samples (i.e., without isolation and culture) remains challenging for viruses such as Zika, for which metagenomic sequencing methods may generate insufficient numbers of viril reads. Here we present a protocol for generating colding-sequence-complete genomes, comprising an online primer design tool, a novel multiplex PCR enrichment protocol, optimized library preparation methods for the portable fillion Sequence (Conford Nanopore Technologies) and the Illiminar page of instruments, and a bioinformatics pipeline for generating consensus sequences. The MinION protocol does not require an Internet connection or analysis, making its studies for fired applications with intinited connectivity, our method relies on multiplex PCR for turgeted enrichment of virial genomes from samples containing as few as 50 genome copies per reaction. Viral consensus sequences and be achieved in 3-2 de y sturing with clinical samples and following a simple laboratory workform. Bin method as belong to successfully used by several groups studying 2lka virus evolution and is facilitating an understanding of the spread of the virus in the Americas. The protocol can be used to sequence other virus igenomes using the online Primaic Scheme primer designer software. It is suitable for sequencing either RNA or DNA viruses in the field during outbreaks or as an inexpensive, convenient method. for use in the lab.

spread of disease in outbreaks¹. Real-time genomic surveil-lance is important in managing viral outbreaks, as it can pro-ide insights into how viruses transmit, spread and evolve¹⁻⁴. Such work depends on rapid sequencing of viral material directly from clinical samples—i.e., without the need to isolate he virus in pure culture. During the Ebola virus epidemic of During recent work on the Zika virus epidemic of the protocol buring recent work on the Zika virus epidemic of the protocol. 2013–2016, prospective viral genome sequencing was able to provide critical information on virus evolution and help inform clinical samples is faster, less laborious and more amenable to
near-patient work than time-consuming culture-based methsequencing, these samples were depleted of human rRNA and near-patient work than time-consuming culture-based methods. Metagenomics, the process of sequencing the total nucleic acid content in a sample (typically cDNA or DNA), has been platform as previously described²³⁷. In these cases, sequences successfully applied to both virus discovery and diagnostics²³⁸, from Zilav irus comprised 20.01% of the data set, resulting the content of the data set, resulting the content of the data set, resulting the data set, resulting the content of the data set, resulting the content of the data set, resulting the data set, re Succession; approaches have seen rapid adoption over the past decade, fueled by refentless improvements in the yield complete coverage. Greater coverage and adequate restaining in Metagenomic approaches have seen rapid adoption over the past decade, fueled by refentless improvements in the yield or sequencing of the past decade, further than the past decade for sequencing of Ebola virus directly from clinical samples with sequencing of Ebola virus directly sequencing and sequencing of Ebola virus directly sequencing and virus copy numbers found in acute cases 13-15. However, direct themselves to the cost-effective use of lower-throughput portable

Genome sequencing of viruses has been used to study the with regard to sensitivity: genome coverage may be low or

epidemiological investigations 3-6. Sequencing directly from These samples had cycle threshold (Ct) values between 33.9 and metagenomic sequencing from clinical samples poses challenges sequencing devices such as the Oxford Nanopore MinION.

NATURE PROTOCOLS | VOL.12 NO.6 | 2017 | 1261





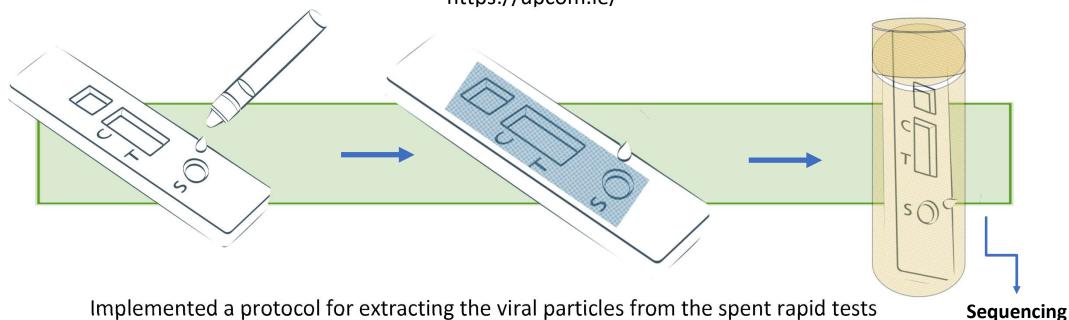
Methods – LFD



SFI-funded project:

Understanding and Preventing COVID-19 Outbreaks in Meat Processing Plants

https://upcom.ie/



Implemented a protocol for extracting the viral particles from the spent rapid tests

Extraction of high quality viral RNA

Direct Sequencing (not needed a second swabs from tested positive)

Detection of variants, tracing outbreaks, surveillance, system for future outbreaks?



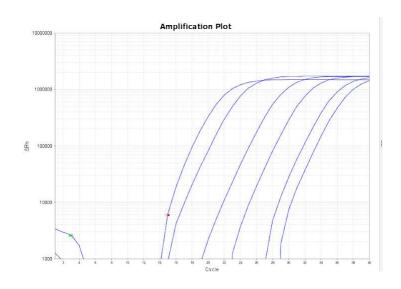
Results – sampling and RT-qPCR



- 10 samples pooled
- Plasmidic DNA
- RNA positive samples

1,261 samples in total

• Process control (MeV) lysis step.



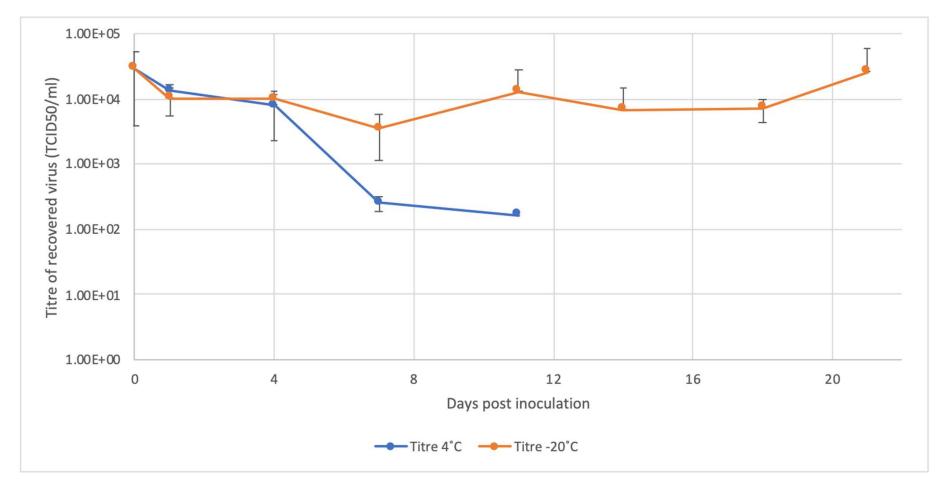
Results

The matrices were extracted and tested in ten units

4 pools resulted very close to the limit of detection

Single samples repeated (40)
& Repeated the extraction from original samples
(31 swabs and 9 fresh vegetables)

All the samples resulted negative



Russell, 2021



Is there a risk from food or packaging?

Guidance from the World Health Organization (WHO) for businesses also states it is highly unlikely that people can contract COVID-19 from food or food packaging.

The risk of getting sick with COVID-19 from eating or handling food, including frozen food and produce and food packages, **is considered very low.** It is possible a person can get it by touching a surface or object, including food or packaging, that has the virus on it and then touching their mouth, nose, or possibly eyes, according to the Centers for Disease Control and Prevention.

National Science Review

Sampling plan: the model

Time for the sampling 3 weeks - 21st January - 11th February

Is this enough?

SOPs Sharing **Networking** caused by other routes of introduction 2-positive environmental samples were and transmission remains unclear, and tested, and 45 were positive by qRTcurrent strategies to prevent resurgence could be flawed. Since July, SARS-CoV-2 To evaluate the extent of infec-RNA contaminations in frozen food tion spreading, a screening campaign of imported from countries with ongoing SARS-CoV-2 infection was implemented epidemics have been reported in nine over the city by Beijing Center for Disprovinces in China [3,4]. However, there ease Prevention and Control. Between and highly clustered cases were identified is no robust evidence of COVID-19 15 June and 10 July, a total of more outbreaks initiated by environment- than 10 million citizens, and 5342 and S3). to-human transmission. Here we add to evidence of such transmission by Eventually 368 qRT-PCR positive investigating the recent COVID-19 resurgence in Beijing. On 11 June 2020, a 52-year old man suffering from fever and cough was XFDM between May 30 and 12 June, diagnosed with COVID-19 in Beijing, after a 56-day zero new case interval. He had no exposure history of known

the places that he had visited were tested

polymerase chain reaction (qRT-PCR). (Fig. S1C).

BRIEF COMMUNICATION

CLINICAL MEDICINE

Special Section: SARS-COV-2

resurgence in Beijing

[1,2], has been contained in China

through stringent non-pharmaceutical

interventions. Border control and quar-

antine have effectively prevented the

virus from being spread by infected

travellers, but the risk of resurgence

environmental samples were screened. cases were confirmed (Fig. S1A), of which 169 (45.9%) had a history of working in XFDM. Of the visitors to 103 (28.0%) were diagnosed. The remaining 96 (26.1%) patients had contact with the infected employees COVID-19 cases. On 12 June, 112 or visitors. These findings suggested a close contacts of the index case and 242 single outbreak source in Beijing (Fig. environmental samples collected from S1B). Retrospective epidemiologiby quantitative reverse transcription symptom onset of a patient on 4 June

Cold-chain food contamination as the possible origin of COVID-19

Xinghuo Pang^{1,2,†}, Lili Ren^{3,4,†}, Shuangsheng Wu^{1,2,†}, Wentai Ma^{5,6,†}, Jian Yang ^{1,2,†}, Lin Di⁸, Jie Li⁹, Yan Xiao^{3,4}, Lu Kang^{5,6}, Shichang Du^{1,2}, Jing Du^{1,2}, Jing Wang^{1,2}, Gang Li^{1,2},

Shuguang Zhai^{1,2}, Lijuan Chen^{1,2}, Wenxiong Zhou⁸, Shengjie Lai¹⁰, Lei Gao⁷, Yang Pan^{1,2,*}, Quanyi Wang^{1,2,*}, Mingkun Li^{5,6,11,*}, Jianbin Wang^{9,12,13,*}, Yanyi Huang ^{®8,14,*}, Jianwei Wang^{3,4,*}, COVID-19 Field Response Group^{1,2} and COVID-19 Laboratory Testing Group^{1,2}

two environmental samples from Xinfadi

Market (XFDM) were positive for SARS-

CoV-2. This led to in-depth investigation

to confirm the role of XFDM in virus

spread. A total of 538 employees from the

booths that were close to the SARS-CoV-

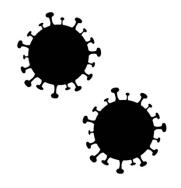
COVID-19, caused by SARS-CoV-2 All close contacts were negative, but

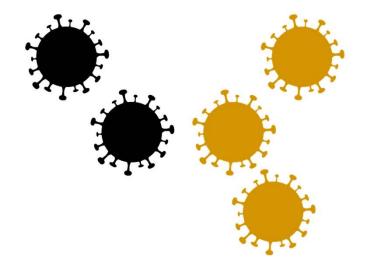
To probe the origin of the infection, we analysed the spatial distribution of infected employees in XFDM. Strikingly, 20.9% (122/584) of employees working in the basement of the XFDM trading hall (XFDM-TH) were positive for SARS-CoV-2, which is significantly higher than those of other areas in the market (1.7%, 47/2727, $\chi^2 = 363.29$, P < 0.001). Meanwhile. their symptom onset dates were also earlier than other employees in the market (Fig. S2). The infections demonstrated spatial clusters in the basement. in the seafood section (Table S1, Figs 1A

We further identified 14 booths (Figs 1A and S4) in XFDM-TH with both employee infections and environmental contaminations, and 3294 individuals who visited these booths from 20 to 31 May. Serological screenings identified five visitors positive for IgG/IgM antibodies against SARS-CoV-2, and they had all been to the booth #S14. In contrast, no other booth was visited by more than two of these five visitors. All five visitors were negcal investigation revealed the earliest ative for qRT-PCR, and none of their close contacts was infected based on qRT-PCR and antibody tests. These

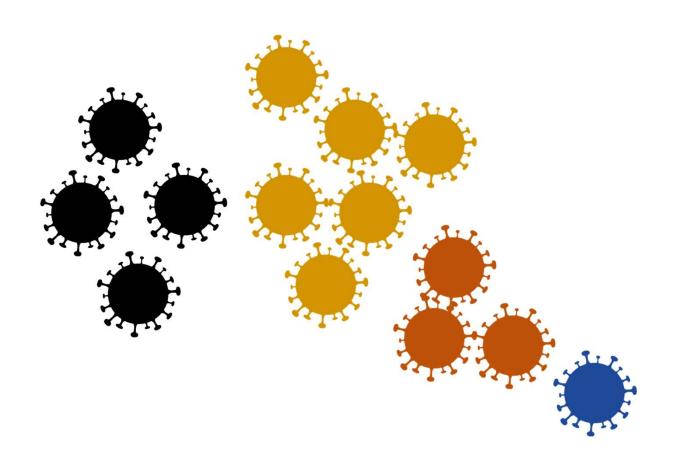
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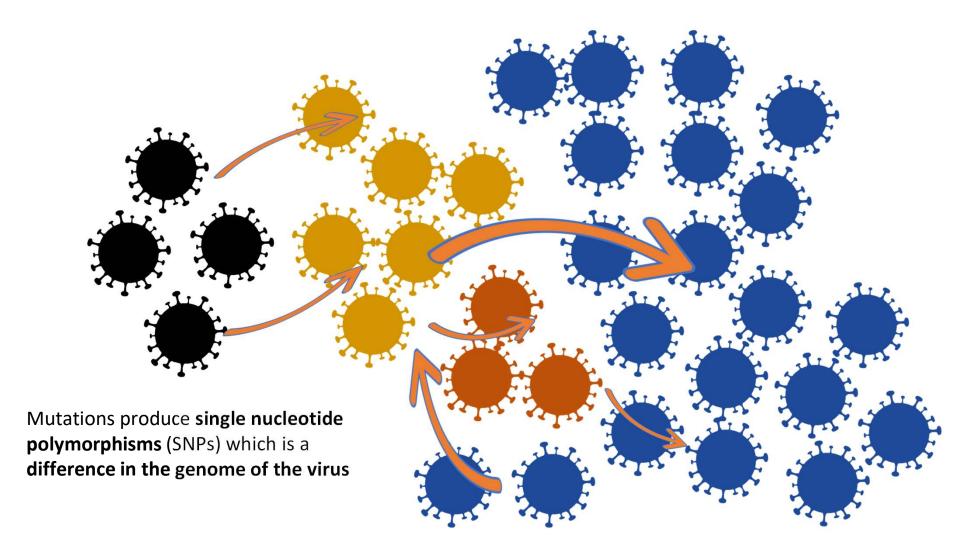




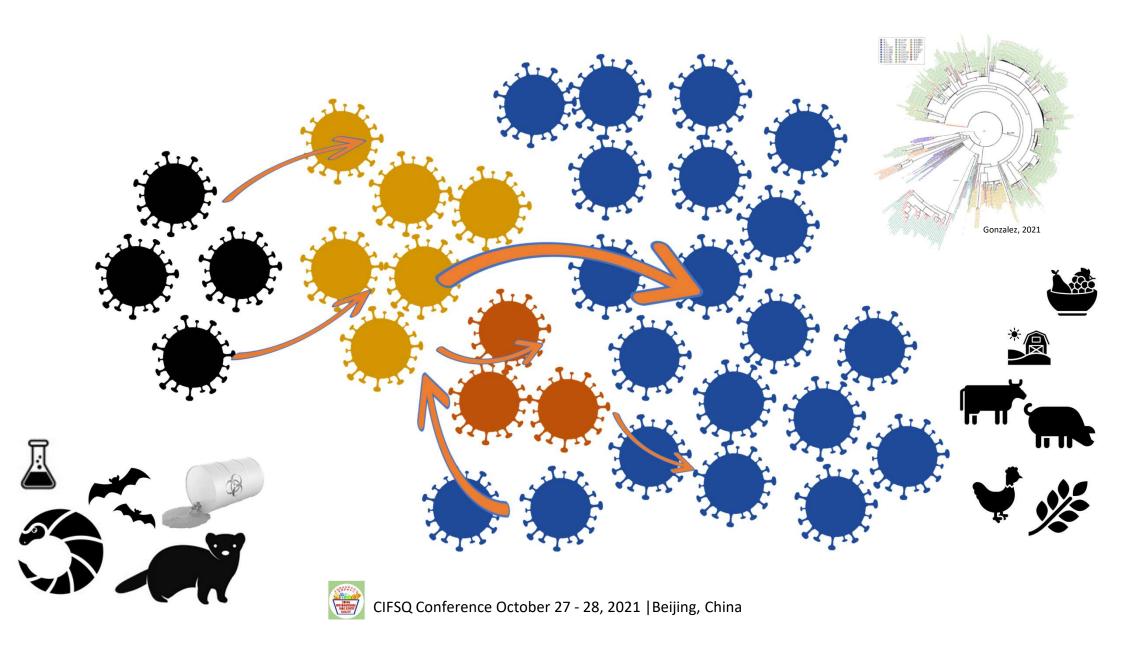


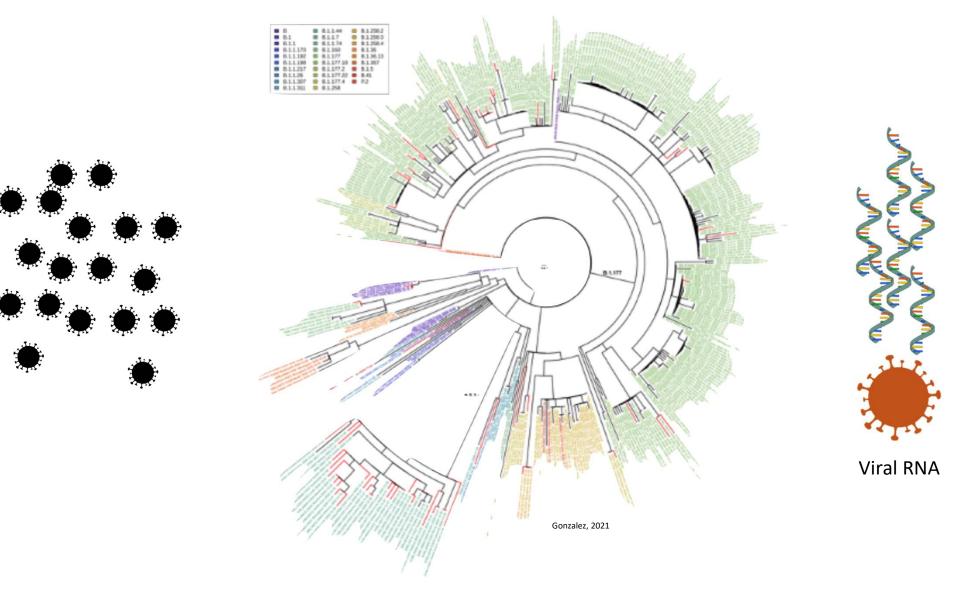




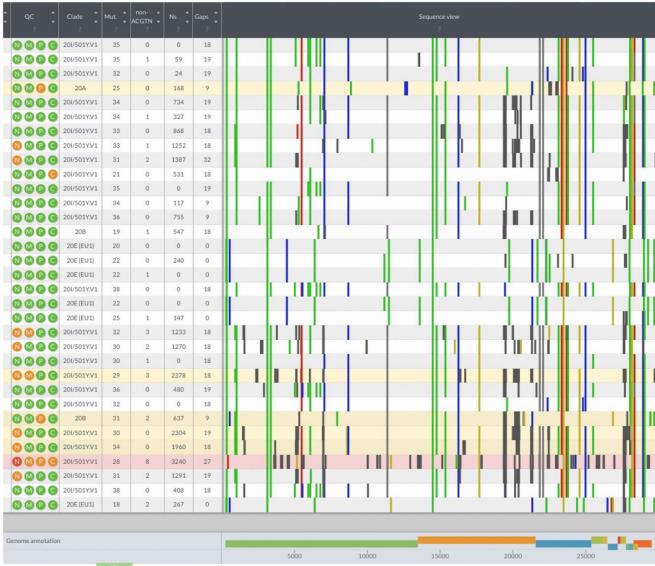






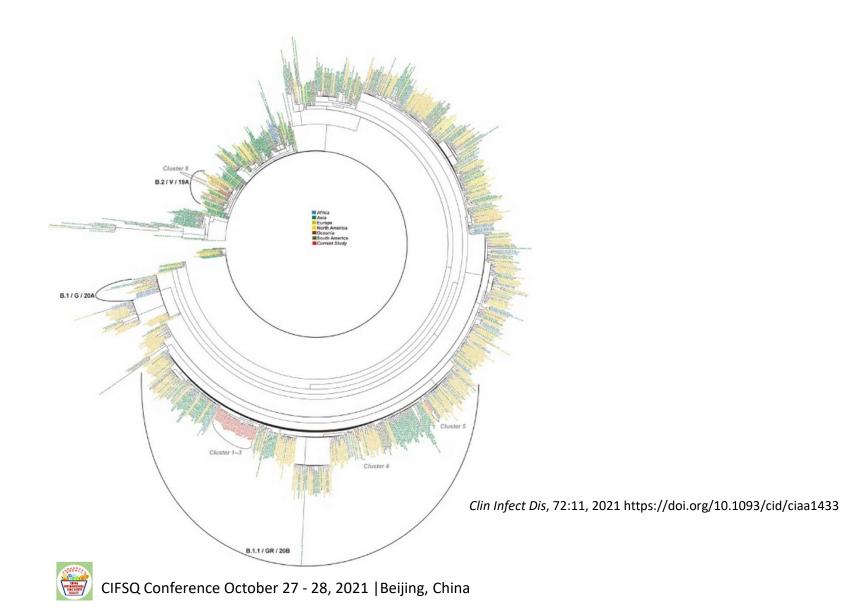














There have been 155 outbreaks of COVID-19 in nursing homes across the country, according to the Health Service Executive (HSE).



COVID-19 has been diagnosed in almost one-third of Ireland's nursing homes as experts warn of a "catastrophe in the making."

There have been 155 outbreaks of COVID-19 in oursing homes across

Seven residents of Belgian nursing home die after outbreak of B.1.621 lineage of COVID-19

1 4 8 8



Los Angeles Times

California names nursing homes with coronavirus outbreaks, number of cases





Report finds staffing and Covid training issues at Louth nursing home where over 20 patients died in April

A report from May - just a week after an outbreak at the nursing home - highlighted some areas of noncompliance at the centre.

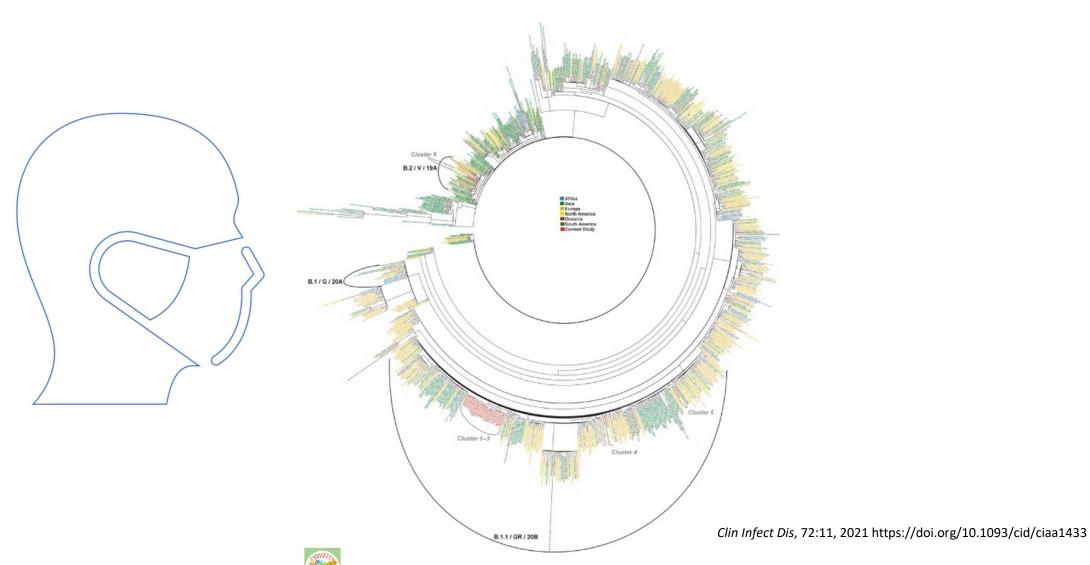


20 nationts died during a Covid-10

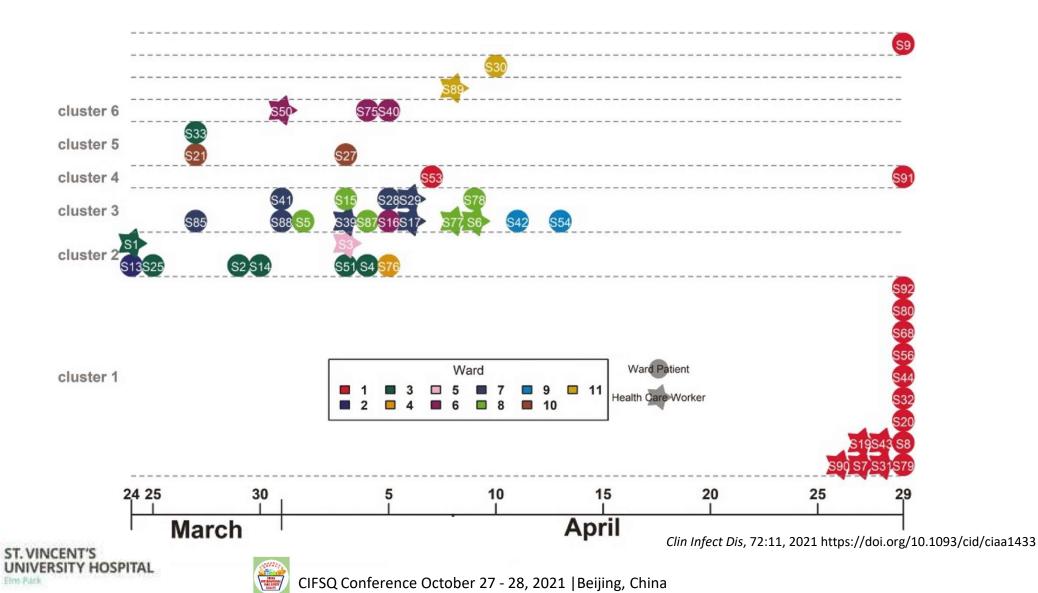
AN INSPECTION REPORT at a

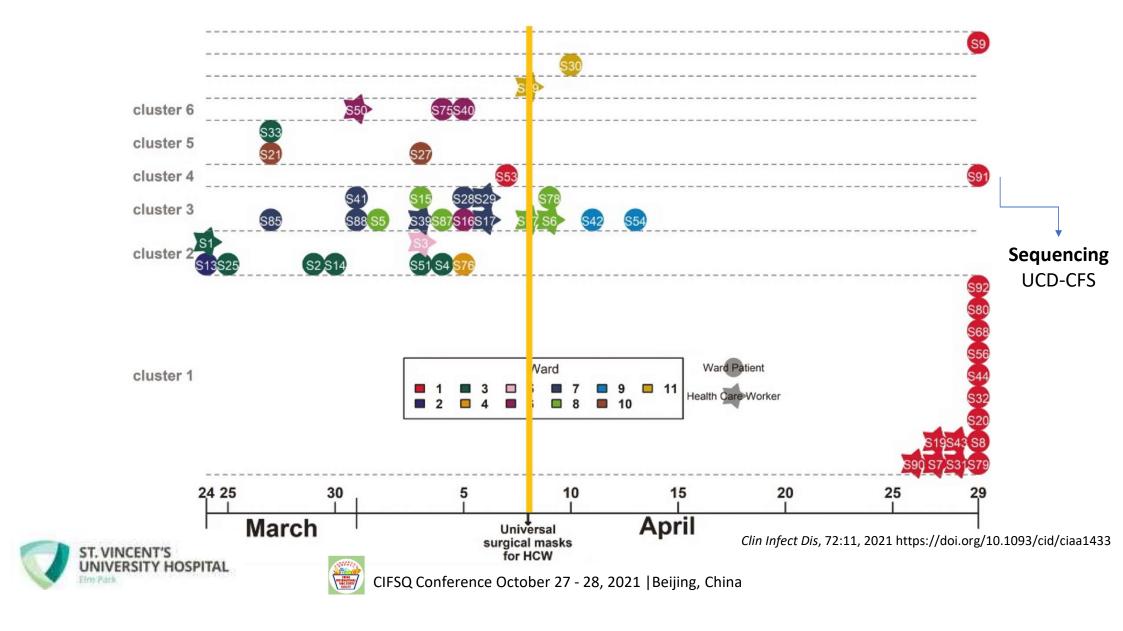






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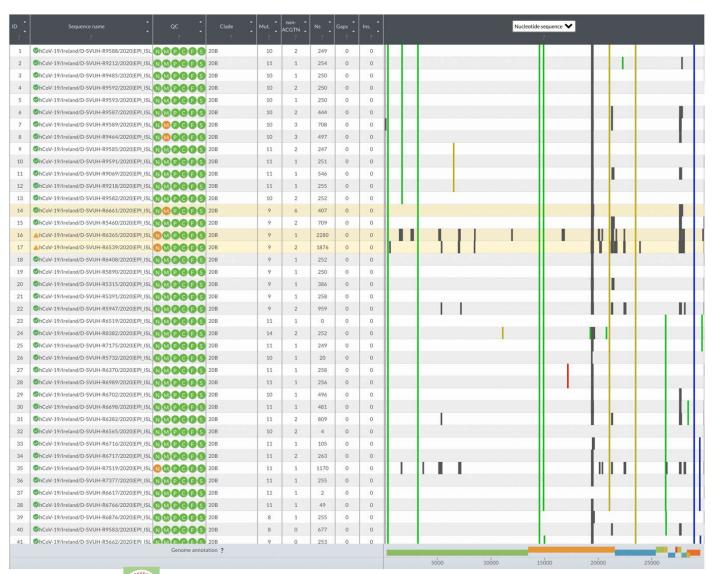




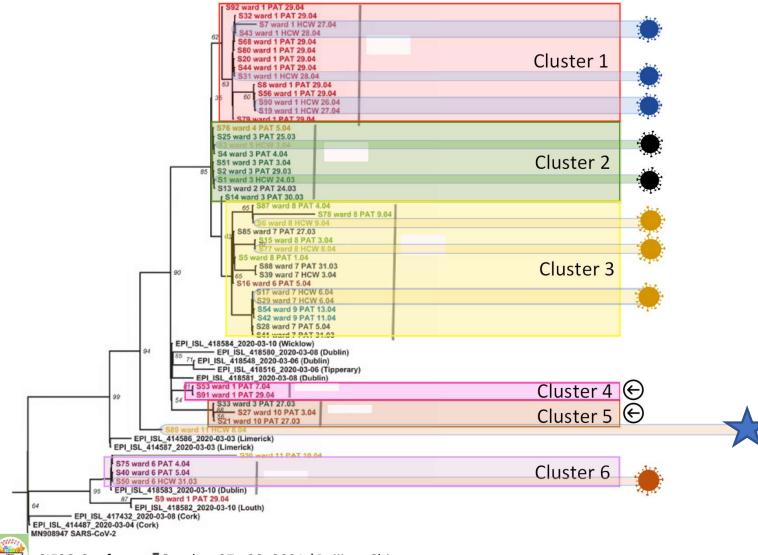
- Health care workers (HCW) close contacts
- Hospital-acquired (HA) COVID-19 cases (patients admitted without symptoms of COVID-19 who had SARS-CoV-2 RNA detected at least 7 days after admission)
- Only patients that were hospitalised for more than 14 days













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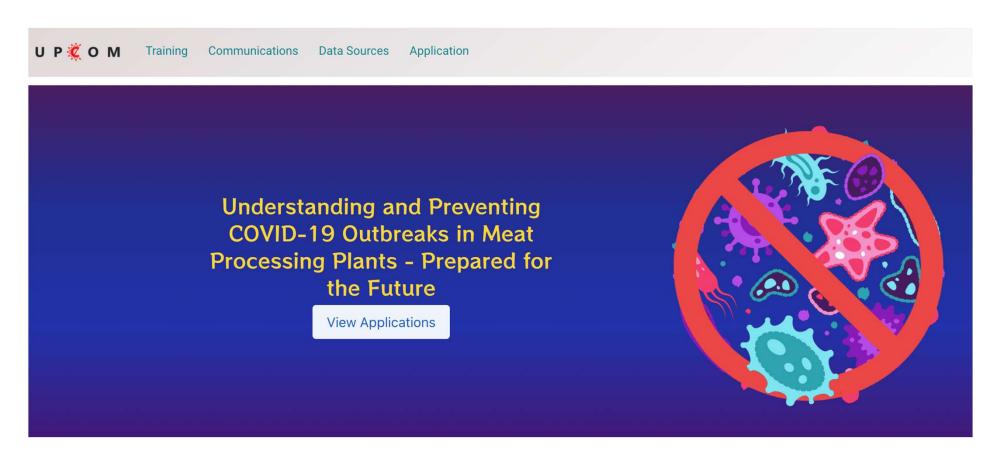




Understanding and Preventing COVID-19 Outbreaks in Meat Processing Plants - Prepared for the Future http://upcom.ie/

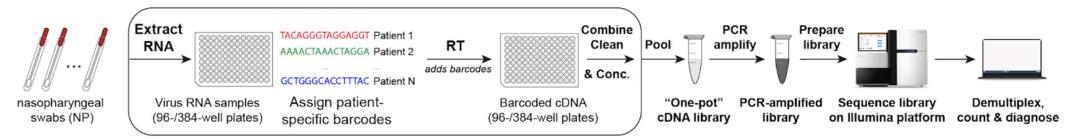


http://upcom.ie/



Massively parallel diagnostic assay (MPDA) platform

- Evaluation of the performances
- Release of a robust prototype



Steps performed in 96/384-well plate formats

Hossain et al., 2020

- Antimicrobial Resistance genes
- Food-borne pathogens
- Sentinels programmes



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...and keep in touch!

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中欧食品安全



guerrino.macori@ucd.ie
@guerrino.macori

