Detection for Novel Pathogen Pandemics



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"Not a single year passes without [which]…we can tell the world: here is a new disease!"

– Rudolf Virchow, *Father of Modern Pathology*, 1867[1]

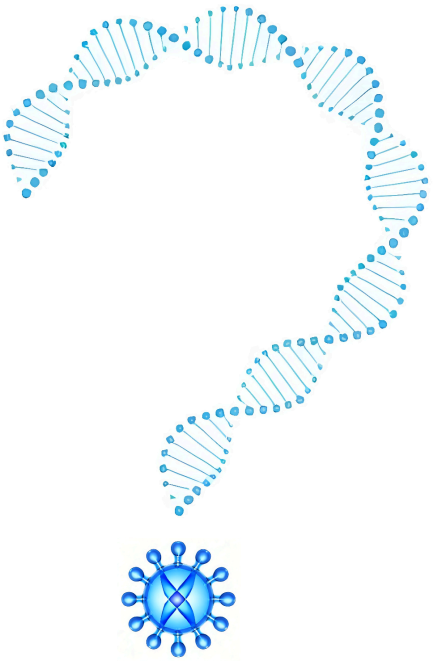
"SARS-CoV-2 is the latest one, but it will not be the last pathogen that breaches the species barrier, so there will be a 'Disease X' in the future. I think we all need to be much better prepared for that."

– Maria Van Kerkhove, *WHO Emerging Diseases and Zoonoses Lead*, 2021[2]

Preface

In early December 2019, when I submitted my *Doctor of Philosophy* report for Transfer of Status a year into my degree, I began by stating, "The next internationally significant infectious disease outbreak could be caused by a currently unrecognised pathogen." Within a month, tragically, this turned out to be true. The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in unparalleled consequences for health, society and economies worldwide. Public health systems and medical facilities around the globe have been strained and, at times, overwhelmed. The detection of the outbreak in Wuhan, China, and later in other regions across the globe, was hampered by the initial difficulty in identifying cases of this novel "Disease X", particularly if they were mild or asymptomatic. In the early stages of the pandemic, the availability of accurate and reliable diagnostic tests was significantly limited, making it difficult to estimate the number of infections in various jurisdictions.

Since the emergence of SARS-CoV-2, the world has gained a wealth of knowledge about this new virus, leading to the development of various tests for its detection, as well as other rapidly deployed countermeasures. However, the statement from my Transfer of Status report still holds true, and we may yet face another pandemic caused by a currently unrecognised pathogen. This raises the question of whether we can be better prepared and detect future unknown biological threats rapidly moving forward. My thesis, initially motivated by this query, and even more so now, examines some of the issues in detecting new diseases and explores potential novel approaches to public health surveillance. My aim has been to contribute to our collective understanding of how we could better utilise early detection and innovations in laboratory science to reduce the likelihood of the next novel pathogen outbreak becoming a global pandemic.



Disease X

Acknowledgements

This thesis would not have been possible without, first and foremost, the love and support of my fiancé Rob. We made it through the COVID-19 pandemic together, and I look forward with excited anticipation to our life going forward.

I am grateful for the advice and support from my supervisors, Mike Bonsall and Robin Thompson. They have shown patience and taught me the lesson of perseverance, and their feedback has been invaluable along this journey. I would also like to thank my colleagues, Amee Manges, Sam Chorlton, and Nick Gauthier, for their gracious input and guidance. Greg Lewis has been a source of optimism and encouragement, and Charlie Whittaker provided indispensable advice and support. In the face of a paralysis limbo, I am eternally grateful for discussions that emboldened me to take action. Conversations with Sophie Rose and Jake Mendel have been enlivening and shown me the value of having good colleagues.

I am also grateful to my family, my parents Troy and Colleen Nelson, who infused me from an early age with a curiosity for science and provided all the support needed and more to see me where I am today. My brother Joshua has been a kindred spirit, Kyla has offered so much love, Tyarra has been an inspiration, Mariah has brought care and understanding, and Breanna has been a constant source of guidance and encouragement through all the good and troubled times. My friends Niel and Lily have offered steady support and an oasis of respite. Ivan has shown me kindness and expert advice. Through conversation, Elise has empowered me. Jade showed me kindness and gave me hope, Jelena provided encouragement, and Dan Epstein and Rob Long have been invaluable conversationalists.

Abstract

Pandemics pose a significant threat to human health and global security. Novel pathogens present substantial pandemic risks and have unique public health challenges, given the uncharacterised nature of their causative agents and the consequent uncertainty about optimal countermeasures. Early detection of novel pathogen outbreaks is crucial for timely public health responses to mitigate devastating consequences. Following outbreak discovery, further detection is essential to ascertain the prevalence and understand the features of the epidemic. This thesis explores different aspects of detection for novel pathogen pandemics, including through a systematic analysis of existing national surveillance infrastructure worldwide for novel diseases. It examines the implications of current surveillance using modelling techniques and investigates diagnostic approaches to determining community infection prevalence in the early stages of a novel pathogen pandemic. We begin by providing an overview of novel pathogens, pandemic risks, and available detection methods, reviewing the literature on “Disease X” and historical case studies of recently emerged pathogens. Our analysis of 195 countries demonstrates that only a minority have evidence publicly available of novel disease considerations in their national public health surveillance systems. For those that mandate novel disease reporting, we model time-to-detection and outbreak size using the detection thresholds specified by different countries and explore whether population-level surveillance strategies could improve upon current detection. A serial cross-sectional investigation of SARS-CoV-2 infection prevalence in a community population in the early stage of the COVID-19 pandemic is presented, with four diagnostic approaches compared. Finally, we summarise our findings in the context of current and future detection for novel pathogen outbreaks and explore research directions that could guide improvements in early warning systems, diagnostic preparedness and pandemic prevention initiatives.

Contributions

All of the chapters and enclosed results, figures and tables that constitute this thesis were written and produced by myself, with input and supervision as listed below.

Chapter Two: Cassidy Nelson (CN), Sophie Rose (SR), Vorathep Sachdev (VS), Robin Thompson (RT), Charles Whittaker (CW), Michael Bonsall (MB): CN designed the study and collected and curated the data. CN developed the methodology and conducted the analysis with supervision from CW and MBB. SR validated the data. CN conducted the literature search and produced the figures and tables. CN wrote the Chapter. CW, SR, VS, RNT, MB and CN revised the Chapter. A paper version of this Chapter is under review after being submitted 12 May 2023 to the *BMJ Global Health,* and is also available as a preprint:

> **Nelson C,** Rose S, Sachdev V, Thompson RN, Whittaker C, Bonsall MB. National Surveillance for Novel Diseases: A Systematic Analysis of 195 Countries.

Chapter Three: Cassidy Nelson (CN), Robin Thompson (RT), Michael Bonsall (MB): CN designed the study and developed the methodology. MB and RT provided supervision. CN conducted the analysis, interpreted the results and produced the figures and tables. CN wrote the Chapter. CN, RT and MB revised the Chapter.

Chapter Four: Cassidy Nelson (CN), Amee Manges,(AM), Nicholas Gauthier (NG), Sam Chorlton (SC), Robin Thompson (RT), Michael Bonsall (MB): CN designed the study and developed the methodology, with supervision from MB. AM, NG and SC provided the RT-PCR and metagenomic sequencing data. CN analysed the data and produced the figures and tables. CN wrote the Chapter. CN, AM, NG, SC, RT and MB revised the Chapter.

Publications

During my studentship, I have contributed as a co-author to peer-reviewed publications that are outside the scope of this thesis.

12. Boyd M, Baker MG, **Nelson C**, Wilson N. The 2021 Global Health Security (GHS) Index: Aotearoa New Zealand's improving capacity to manage biological threats must now be consolidated. The New Zealand Medical Journal. 2022 Aug 19;135(1560):89-98. ❄

11. Chandrakumar I, Gauthier NP, **Nelson C**, Bonsall MB, Locher K, Charles M, MacDonald C, Krajden M, Manges AR, Chorlton SD. BugSplit enables genome-resolved metagenomics through highly accurate taxonomic binning of metagenomic assemblies. Communications Biology. 2022 Feb 22;5(1):151. ❄

10. Gauthier NP, **Nelson C**, Bonsall MB, Locher K, Charles M, MacDonald C, Krajden M, Chorlton SD, Manges AR. Nanopore metagenomic sequencing for detection and characterization of SARS-CoV-2 in clinical samples. PLoS ONE. 2021 Nov 18;16(11):e0259712. ❄

9. Sutherland WJ, Taylor NG, Aldridge DC, Martin P, Rhodes C, Shackelford G, Beard S, Belfield H, Bladon AJ, Brick C, Burgman M, Christie A, Dicks L, Dobson A, Downey H, Hood A, Hua F, Hughes A, Jarvis R, MacFarlane D, Morgan W, Mupepele A, Marciniak S, **Nelson C**, Ó hÉigeartaigh S, Roja C, Savolainen R, Sainsbury K, Smith R, Sundaram L, Tankard H, Thornton A, Watkins J, White T, Willot K, Petrovan S. A solution scan of societal options to reduce transmission and spread of respiratory viruses: SARS-CoV-2 as a case study. Journal of Biosafety and Biosecurity. 2021 Dec 1;3(2):84-90. ❄

8. Lewis G, Jordan JL, Relman DA, Koblentz GD, Leung J, Dafoe A, **Nelson C,** Epstein GL, Katz R, Montague M, Alley EC, Filone C, Luby S, Church G, Millett P, Esvelt K, Cameron E, Ingelsby T. The biosecurity benefits of genetic engineering attribution. Nature Communications. 2020 Dec 8;11(1):6294. ❄

7. Boyd MJ, Wilson N, **Nelson C**. Validation analysis of global health security index (GHSI) scores 2019. BMJ Global Health. 2020 Oct 1;5(10):e003276. ❄

6. Boyd M, Baker MG, **Nelson C**, Wilson N. The 2019 Global Health Security Index (GHSI) and its implications for New Zealand and Pacific regional health security. The New Zealand Medical Journal. 2020 Jun 12;133(1516):83-92.

5. O'Brien JT, **Nelson C**. Assessing the risks posed by the convergence of artificial intelligence and biotechnology. Health Security. 2020 Jun 1;18(3):219-27.

4.Sandberg A, **Nelson C**. Who should we fear more: biohackers, disgruntled postdocs, or bad governments? A simple risk chain model of biorisk. Health Security. 2020 Jun 1;18(3):155-63.

3. Kemp L, Adam L, Boehm CR, Breitling R, Casagrande R, Dando M, Djikeng A, Evans NG, Hammond R, Hills K, Holt LA, Kuiken T, Markotić A, Millett P, Napier JA, **Nelson C**, Ó hÉigeartaigh S, Osbourn A, Palmer MJ, Patron NJ, Perello E, Piyawattanametha W, Restrepo-Schild V, Rios-Rojas C, Rhodes C, Roessing A, Scott D, Shapira P, Simuntala C, Smith RDJ, Sundaram LS, Takano E, Uttmark G, Wintle BC, Zahra NB, Sutherland WJ. Bioengineering Horizon Scan 2020. Elife. 2020 May 29;9:e54489.

2. **Nelson C**, Franklin L, Gibney K. Infectious Disease Notification Practices in Victoria, 2016-17. Communicable Diseases Intelligence. 2019 Aug 15;43:1-16.

1. MacIntyre C, Heslop D, Nand D, Schramm C, Butel M, Rawlinson W, Baker M, Kiedrzynski T, **Nelson C**, Rosewell A, Fotualii L, Yeo K, Elsgaard J, Fonua L, Lane JM. Exercise Mataika: White Paper on response to a smallpox bioterrorism release in the Pacific. Global Biosecurity. 2019 Feb 14;1(1):91–105.

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1

Introduction

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Pandemics pose a substantial threat to human health, global security and economies.[3] Novel pathogens constitute a significant portion of the pandemic risk, as evidenced by the fact that nearly all recent outbreaks of international concern have been caused by newly emerged infectious diseases.[4] Early detection of novel pathogen outbreaks with pandemic potential is

of paramount importance to all countries, but surveillance is impeded by numerous "unknown unknowns" related to their emergence.[5] In this Chapter, we provide an overview of the literature on novel pathogens and the pandemic risk they confer, examine existing detection methods, and present historical case studies of new disease emergence to consider the potential barriers to the timely identification of outbreaks.

Section 1.1 Novel Pathogens

Novel pathogens are infectious biological agents capable of causing disease in their hosts that have previously been unknown or unrecognised. Such pathogens constitute one aetiological subset of emerging infectious diseases (EIDs), defined as "infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range."[6] Since 1940, pathogens have been emerging with increasing frequency due to a variety of social, economic, environmental and ecological factors, despite improvements in infectious disease surveillance during this time period.[7] Zoonotic reservoirs are the main contributor to EIDs entering human populations, with the majority of new pathogens originating from wildlife.[8] A 2022 study found that bats are a substantial contributor of emerging viruses capable of infecting humans and that with current and projected changes in the climate and land use, model results predict 4,584 (±52) novel viruses will cross the mammalian species barrier by 2070.[9] Not all of these pathogens will pose a pandemic risk to humans, as outbreaks require both pathogen introduction to human hosts and establishment of the disease in the population with sustained transmission.[10] While the risks of EIDs are widely acknowledged, several challenges remain in the early detection of novel pathogen spillover events and the timely identification of new disease outbreaks.[5,10,11]

In 2007, a study examining the 1,399 pathogens capable of infecting humans known at the time found that 87 (6.2%) of these species were new, having been discovered from 1980 onwards.[12] While new infectious agents can come from various taxonomic categories, including viruses, bacteria and fungi, this paper noted that viruses constitute a disproportionate 58/87 (66.7%) of newly discovered pathogens, with 49/58 (84.5%) of these being RNA viruses. This is partially due to the accelerated evolution that most viral RNA genomes undergo because of their increased mutation rates during replication and reverse transcription.[13,14] A prototypical pathogen in this category is influenza, a single-stranded RNA virus that undergoes rapid evolution in human and animal populations, producing new subtypes that result in annual epidemics.[15]

In addition to natural emergence through evolutionary processes and zoonotic spillover events, developments in biotechnology and synthetic biology have raised the concern that genetically engineered pathogens may also pose an increasing novel disease threat this century if such agents were ever accidentally or deliberately released.[16,17] Laboratories are a notable setting where humans can come into contact with infectious agents. Accidents in these environments involving pathogen exposure are not uncommon, with 71 high-risk events reported between 1975 - 2016.[18] However, as the authors of that study note, there can be significant missing data problems in laboratory accident reporting. Therefore, this figure is likely a lower bound. The risks from novel pathogens may increase in coming decades due to accelerating capabilities in artificial intelligence and gene editing technology such as CRISPR-Cas systems allowing for the accidental or deliberate design of infectious agents with enhanced pathogenicity.[19–21] The potential threats posed by advances in biotechnology have led to calls for improved research governance on the manipulation and creation of novel pathogens with pandemic potential.[17,22]

In addition to laboratory accident risks, deliberate biological events could pose an increasing pandemic risk as the barriers to entry and difficulty in pathogen design decrease.[23] There is already a long-standing history of state and non-state actor interest in biological weapons and bioterrorism.[24,25] However, recent developments in biotechnology have raised the concern of opportunities for more advanced novel biological weapon creation than in the past.[26–28] Such pathogens, if released, could pose a global catastrophic biological risk (GCBR), defined as a biological event leading to widespread disaster beyond the collective capability of national governments and international institutions to control.[29] Engineered pathogens may pose a greater GCBR threat than naturally occurring pandemics, given they could potentially be designed with higher transmissibility and virulence than their counterparts found in nature.[30]

Section 1.2 Pandemic Risk

Novel pathogens which transmit between hosts can result in an epidemic, defined by a rapid rise in the number of infections in the population. Whether a pathogen emergence will result in a minor or major epidemic can be estimated from the basic reproductive number ($\mathcal{R}_0$) and the number of initial infections (I_0).[31] In this context, a minor epidemic is defined as an outbreak which rapidly dies out, while a major epidemic occurs if there is sustained growth in the number of infections. The I_0 depends on the scenario, including the source and number of people exposed. $\mathcal{R}_0$ is an epidemiological metric that quantifies the average number of secondary cases that each infected person will generate during the period of communicability in which they are infectious.[32] When the $\mathcal{R}_0$ is greater than one, the epidemic is expected to grow, while when it is less than one, it will become extinct over time. An epidemic which spreads worldwide becomes a pandemic, although the exact geographical criteria differ, and there is no precise definition of when an epidemic becomes a pandemic.[33]

Epidemics caused by previously unrecognised pathogens present unique public health challenges compared with outbreaks of known diseases. When a novel pathogen first emerges, the causative agent will not have been characterised, and the symptom profile will be undefined. This can impede diagnosis and lead to delays in outbreak detection.[34,35] Only after detection takes place can public health officials form a case definition, the symptom and laboratory criteria by which a person can be assessed to determine whether or not they have the disease.[36] Countermeasures, including accurate diagnostic tests, vaccines and therapeutics, are unlikely to be readily available when a disease first emerges.[37] Furthermore, the background population may not have any pre-existing immunity to the new disease. However, partial cross-immunity may be present if the new disease is a variant of a previously circulating pathogen.[38] Novel diseases pose a significant pandemic risk, in part due to these features, with most recent outbreaks of international concern caused by newly emerged pathogens.[4]

Acknowledging the potential for a currently unrecognised pathogen to cause a public health emergency of international concern, in 2018 the World Health Organisation (WHO) added "Disease X" to its priority list for further research and development efforts.[39] As defined by the WHO, Disease X is not a particular disease but instead represents any unknown pathogen that may pose a significant disease threat to humans, or a recognised biological agent which has undergone changes to its epidemiological characteristics that increase its pandemic risk, such as the evolution of new pathogenic traits resulting in increased transmissibility or disease severity. There are various challenges for pathogen-specific technologies to be deployed during Disease X outbreaks, leading to calls for pathogen-agnostic technologies to be prioritised and their regulatory pathways accelerated.[40,41]

Section 1.3 Detection

Early detection of future Disease X events is critical for implementing timely and effective public health interventions.[42] Identification that an outbreak has commenced is a necessary prerequisite for public health authorities to allocate investigation resources and conduct targeted interventions. In addition, for the 196 signatory countries of the International Health Regulations (2005), it is mandatory to notify the WHO of "any event of potential international public health concern, including those of unknown causes."[43] There are various mechanisms that can be used for novel infectious disease surveillance. Broadly, these can fall under three categories: Event-based,indicator-based and community-based surveillance, as summarised in Table 1.1.

Table 1.1 Summary overview of different surveillance strategies.

<table>
<tr><th colspan="2">Event-Based Surveillance</th><th>Indicator-Based Surveillance</th></tr>
<tr><td colspan="2" rowspan="2">1. Event Surveillance
Can use a variety of information sources and datastreams, including social media, local news, internet search terms, pharmacy purchases, wearable devices, community rumours and other forms of unstructured data
Not disease- or symptom-specific
Can theoretically detect disease events more rapidly than traditional methods
Requires timely data monitoring and analysis
Potential for generating false positives</td><td>2. Disease-Specific Surveillance
Traditional surveillance relying on mandated reporting from doctors and laboratories
Requires knowledge of the disease, including specified tests or clinical criteria</td></tr>
<tr><td>3. Syndromic Surveillance
Also relies on mandated reporting, but monitors symptoms and syndromes instead of defined disease diagnoses
Can potentially detect novel diseases that do not have definitive test/clinical criteria</td></tr>
<tr><th colspan="3">Community-Based Surveillance</th></tr>
<tr><td colspan="3">4. Community Surveillance
Reporting of community public health events by members of that community, such as trained community health workers or volunteers
Typically incorporates event-based surveillance elements, but can include event-based or indicator-based surveillance, or a combination of both
Particularly useful in rural, lower-resource or crisis settings
Can lead to early detection and reporting of outbreaks, as well as ongoing event monitoring</td></tr>
</table>

Event-based surveillance (EBS) is a proactive approach to detecting emerging disease threats by monitoring unusual events, rumours, and other sources of information.[44] For example, the first signals of an outbreak could come from informal reports of illness or death, or be detectable through analysing data from wearable devices or the use of internet search terms.[45,46] EBS has the potential to identify novel pathogen outbreaks before they are detected through other methods. In countries with limited public health infrastructure, EBS is particularly valuable in its ability to provide an early warning of disease events as reporting through medical facilities may be delayed.[47] However, the 2021 Global Health Security Index (GHSI) found only 89/195 (45.6%) nations operate any form of EBS.[48] Furthermore, the 2021 GHSI found only 18/195 (9.2%) countries conduct daily EBS data analysis, reducing the timeliness of these systems. A 2022 study found that poor data quality, delayed analysis and the low frequency of reports impair the timeliness of EBS, particularly in low- and middle-income countries.[49] Even when real-time data analysis is present, there remain several challenges and limitations that have hindered the widespread adoption and effectiveness of EBS.[50]

Indicator-based surveillance (IBS) relies on reports from health professionals and laboratories, typically through mandating notification of a specified list of diseases or syndromes.[51] This can also include obligations to report suspected cases of new or unknown diseases. Because disease-specific IBS usually requires specified clinical and laboratory diagnostic criteria, which novel pathogens inherently lack, syndromic surveillance is more readily utilised for the detection of unknown disease outbreaks.[52] In the context of early outbreak detection, IBS is inherently limited by the fact that this surveillance mechanism usually requires symptomatic patients to present to a health service.[53] This means that when a new disease causes at least some asymptomatic or mild illness in a proportion of those

infected, the first clusters of cases may not be initially identified. Underreporting of cases to public health authorities can also undermine the timeliness and effectiveness of IBS systems.[54]

Community-based surveillance (CBS) refers to the reporting of public health events by community members, often including volunteers or community health workers. Although CBS typically incorporates elements of EBS, it can encompass either EBS, IBS, or a combination of both when used for infectious disease outbreak detection.[55] CBS can prove especially beneficial in rural communities lacking public health infrastructure or medical facilities, as well as in low-resourced and crisis settings. For CBS to effectively detect outbreaks at any early stage, it requires active participation from the community, robust training, and timely integration of reporting into wider surveillance systems.[55]

Early outbreak detection, which can strongly aid in containing an epidemic and reducing its overall size, can be significantly hindered for new diseases which do not have symptom criteria or laboratory tests available to assist in diagnosis.[42] Determining a sensitive case definition, through which suspected cases could be assessed against criteria to determine whether they have the disease, has been historically difficult for new infectious diseases. For example, in 2003, the WHO case definition for Severe Acute Respiratory Syndrome (SARS) based on symptom criteria had only a 26% (95% Confidence Interval [CI]: 17 - 36%) sensitivity, indicating a large proportion of false negatives that risked truly infected cases being misclassified as not having the disease.[56]

Widely used laboratory diagnostic tests for disease outbreaks, such as reverse-transcription polymerase chain reaction (RT-PCR) assays, are pathogen-specific and require knowledge of

a predetermined gene target.[4] This limits their utility in the early stages of a novel disease outbreak, where the biological agent has yet to be characterised and its genome is unknown. Other tools, such as serological assays to detect disease-specific antibodies, also require knowledge of the pathogen and the humoral immune response it causes to determine the diagnostic target. The development of sensitive and specific serological assays against new emerging diseases can face several challenges, including overcoming issues with potential cross-reactivity of antibodies and test validation.[57]

Newly developed laboratory tools, however, could potentially overcome some of these barriers. For example, metagenomic next-generation sequencing (mNGS) technologies can sequence all the genetic material in a sample in a hypothesis-free fashion.[58] This approach can provide agnostic detection of new pathogens and remain robust in the presence of gene mutation. mNGS was successfully used to diagnose patients presenting with unexplained pneumonia with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection early in 2020 in China.[59,60] Since then, mNGS tools have continued to improve and researchers have demonstrated the ability to detect SARS-CoV-2 in samples with high sensitivity and specificity even when reference sequences are unavailable.[61] While significant technical challenges remain to the widespread adoption of this technology, its unique ability to detect novel pathogens without *a priori* knowledge of its genome demonstrates significant promise in its utility as a pathogen-agnostic surveillance tool.[62–65] In addition, mNGS could reduce the time between novel pathogen discovery and genetic characterisation, given its ability to reconstruct full-length viral genomes even when the agent was previously unknown.[66] This could potentially accelerate the development and deployment of countermeasures that require genome information, such as RT-PCR tests. There have been

calls for incorporating mNGS into global surveillance for new outbreak detection and reducing the time from discovery to pathogen characterisation.[67,68]

1.3.1 Benefits of Early Detection

To decrease pandemic risk, early detection of a novel pathogen outbreak is crucial for implementing timely and effective public health interventions.[42] Rapid identification of a new disease and recognition that an epidemic has started provides various benefits. Firstly, it allows public health authorities to prioritise resource allocation and implement targeted interventions in affected areas. This can include mobilising medical personnel, distributing personal protective equipment, and implementing public health orders in local communities. Public health officials need to respond proactively before the outbreak has grown too large for measures such as isolation, quarantine, and contact tracing to remain effective.[69] Such response activities can help to limit the spread of the pathogen and reduce the overall number of infections. Local containment may be possible with these measures alone or may also require social distancing, depending on the proportion of asymptomatic and pre-symptomatic infectious cases.[70] If successful, by controlling the outbreak at an early stage, public health authorities can mitigate the impact on healthcare systems and minimise the need for more severe restrictions such as national lockdowns or travel bans.

Secondly, the discovery of the outbreak is a necessary prerequisite to understanding the disease characteristics and transmission dynamics. Even when containment is not possible, early detection facilitates pathogen characterisation and the rapid development and deployment of diagnostic assays, which are critical for confirming cases and monitoring the pathogen's spread.[71] Additionally, identification of the pathogen allows researchers to begin studying its genome and characteristics and investigating potential therapeutics or vaccines.[39]

Early detection accelerates the overall process of countermeasure development and deployment, ultimately saving lives and reducing the burden on healthcare systems.

Thirdly, when an outbreak is detected early, health authorities can more quickly communicate accurate information about the disease, its symptoms, and prevention measures to the public.[72] Risk communication can help dispel misconceptions, reduce panic, and encourage individuals to take appropriate health precautions, such as handwashing and social distancing. Clear and transparent communication is essential for maintaining public trust and ensuring cooperation with public health measures. Social cohesion against the disease threat could enable better coordination between local and national governments and international public health organisations.[73] Such cooperation may lead to a more unified global response, sharing of resources and expertise, and ultimately prevention of outbreaks becoming pandemics.

Section 1.4 History of Emergence Detection

Despite the benefits of early detection, EID epidemics can face significant discovery delays, with a study finding the global median time from outbreak start to detection to be 20 days (95% CI: 16–25 days) and to laboratory confirmation to be 36 days (95% CI: 32–46 days) between 1996 - 2014.[74] However, this study examined all EIDs, and not just ones caused by previously unknown pathogens. Understanding discovery delays for novel disease outbreaks is important to determine potential solutions that could lead to earlier detection.

Over the last fifty years, there have been several examples of new disease emergence that demonstrate some of the reasons for delay and difficulties faced in outbreak detection and identification of novel pathogens. Six case studies of new disease emergence, and the steps

leading to their detection, are outlined below. Figure 1.1 shows the country location and year that each outbreak was first discovered, which can differ from the time and place of pathogen characterisation and retrospectively identified true emergence.

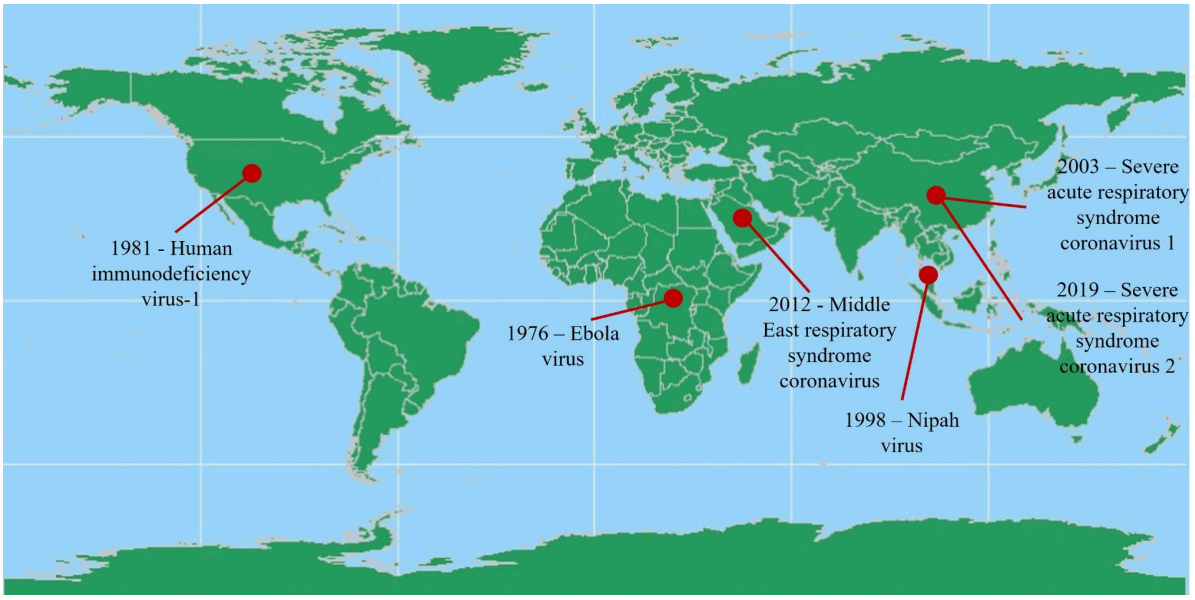


Figure 1.1 World map showing six case study examples of novel disease emergence in the last 50 years, with the country and year of outbreak discovery depicted along with the name of the pathogen. Note the location and time of first detection can differ from where and when the disease was later found to have truly emerged and the time and place the pathogen itself was characterised.

1.4.1 Ebola Virus Disease

Ebola virus disease first emerged in 1976 in what is now the Democratic Republic of the Congo (DRC) and South Sudan.[75,76] The outbreak was first reported on 21 September 1976 in the DRC, at which stage there had been 17 cases of an ”unknown disease.”[77] The first identified case was an adult male who presented with fever on 26 August 1976 to an outpatient clinic and was initially treated for suspected malaria.[78] He died on 8 September 1976. Clinical samples taken on 28 September 1976 were sent to three laboratories around the world, with a novel virus isolated on 13 October 1976. A WHO team was sent to Sudan on 29 October 1976 to investigate reports of haemorrhagic fever in two towns retrospectively identified cases dating back to 27 June 1976.[75] The outbreak was declared over on 16

December 1976, after a total of 318 cases and 280 deaths in DRC and 284 cases and 151 deaths in South Sudan.[75,76]

1.4.2 Acquired Immune Deficiency Syndrome

The first report of what would come to be called Acquired Immune Deficiency Syndrome (AIDS) was released on 5 June 1981 by the Centers for Disease Control in the United States, detailing five unusual cases of *Pneumocystitis carinii* in previously healthy men where a new unknown form of cellular-immune dysfunction was suspected.[79] The onset of symptoms in the first case was in September 1980, with the first hospitalisation in February 1981. However, it was not until 20 May 1983 that the causative agent, Human Immunodeficiency Virus-1 (HIV-1), would be isolated and characterised from a lymph node biopsy in France.[80] Subsequent investigations have traced the first laboratory-confirmed case of HIV-1 back to a 1959 sample from the DRC.[81] By 2019, there had been at least 39 million HIV/AIDS deaths and more than 36 million persons living with HIV, with two million new cases per year.[82]

1.4.3 Nipah Virus Infection

Nipah virus infection is a zoonotic disease which causes fever and acute encephalitis. The first documented Nipah virus outbreak occurred in Malaysia, with the symptom onset in the first detected case on 29 September 1998.[83] The first outbreak cluster consisted of 28 people who had been in close contact with pigs becoming ill with encephalitis and respiratory symptoms. The cause was initially attributed to Japanese Encephalitis due to positive IgM samples in four of the 28 individuals, of whom 15 died.[84] After two further clusters of human cases, including in neighbouring Singapore with similarly high fatality rates, a novel pathogen was suspected and further investigations were undertaken starting 27 February 1999.[85] A novel paramyxovirus was discovered on 9 March 1999.[86] The outbreaks in

Malaysia and Singapore were contained by 27 May 1999 after 276 cases and 106 deaths between the two countries in total.[87]

1.4.4 Severe Acute Respiratory Syndrome

The 2002-2003 Severe Acute Respiratory Syndrome (SARS) outbreak was first detected in two cases on 2 January 2003 in Heyuan, Guangdong, China.[88] The first death occurred on 23 January 2003. On 3 February 2003, a standard symptom-based case definition was issued in Guangdong Province, China. Through retrospective case finding, the earliest identifiable case was found to have first developed symptoms consistent with SARS on 16 November 2002 and presented to the hospital on the same day. SARS spread from China to Vietnam and Hong Kong, with the WHO issuing a global alert on "atypical pneumonia" on 12 March 2003.[89] After further spread to a total of 30 countries and territories, the outbreak was finally contained in July 2003, with 8,437 cases and 813 deaths worldwide attributed to SARS.[90]

1.4.5 Middle East Respiratory Syndrome

Middle East respiratory syndrome coronavirus (MERS-CoV) is a respiratory virus with a high fatality rate typically spread through contact with camels and limited human-to-human transmission.[91] The first known case of MERS was a Saudi Arabian man who presented to the hospital with respiratory symptoms on 13 June 2012 and subsequently died.[92] The case was first reported by the treating physician on 18 June 2012.[93] The patient had returned negative tests for various known pathogens. After clinical sample sharing with the Netherlands, the recognition of a novel coronavirus as the cause of the patient's illness was first published on ProMED on 20 September 2012.[94] Since then, sporadic cases with human-to-human transmission, including an outbreak in South Korea in 2015, have resulted in a total of 2,499 confirmed cases and 858 deaths globally as of December 2019.[95]

1.4.6 Coronavirus Disease 2019

The Coronavirus Disease 2019 (COVID-19) pandemic, caused by the novel SARS-CoV-2 virus, had cases first detected in Wuhan, China. The outbreak was reported on 30 December 2019 in four patients who had been admitted to the hospital with "pneumonia of unknown aetiology" through an alert to the Wuhan Centre for Disease Control, after which the cluster was reported to China CDC and a post uploaded to ProMED to inform the international community.[96] By 3 January 2020, a novel coronavirus related to SARS-CoV-1 had been identified.[97] The earliest symptom onset date was determined to be 1 December 2019 and the earliest hospital admission 16 December 2019.[98] The first death attributed to SARS-CoV-2 occurred on 9 January 2020.[99] Unfortunately, by the time of detection, the virus had already spread beyond the point of containment and subsequently led to the global COVID-19 pandemic.

The above case studies exemplify some of the difficulties in novel disease outbreak detection. In many instances, there was a delay between the first case presentation to a medical facility and reporting to public health authorities, by which time the outbreak had increased in size. Sometimes, as was the case in SARS-CoV-1 and HIV-1, the first outbreak cases were only retrospectively discovered. Diagnostic misidentification can also lead to delays in detecting a novel disease outbreak, such as in the case of Ebola and Nipah virus.[77,84] Even when detection occurs in the first known case, as occurred with the emergence of MERS, there can be a delay to pathogen characterisation that inhibits the timely reporting of a novel disease.[94]

Not all of these emergent events posed a pandemic risk given the pathogen could have low transmissibility, with the $\mathcal{R}_0$ of MERS-CoV ranging from 0.50 - 0.92 and Nipah virus from 0.75 - 0.88.[100,101] Because the $\mathcal{R}_0 < 1$ for these diseases, this means that for each infected case,

on average, less than one new case will occur. However, the authors of these studies note that future strains of these viruses could pose a pandemic risk if they acquire the capacity for more efficient human-to-human transmission.[100,101] The $\mathcal{R}_0$ values of SAR-CoV-1 and SARS-CoV-2 when they first emerged have been estimated at 2 - 3.3 and 3.28, respectively, but the latter had greater pandemic potential in part due to its increased propensity for mild and asymptomatic transmission.[102,103] Together, these case studies provide an imperative to investigate time-to-detection and whether early warning systems for outbreaks can be improved when new pathogens once again emerge.

Section 1.5 Thesis Outline

This thesis explores some of the main issues in detection for novel pathogen outbreaks that have the potential to become pandemics. Our aim was to determine the current state of novel pathogen surveillance globally and its implications, and explore potential future approaches to early detection. Each chapter explores different angles on this topic, as outlined below.

In **Chapter Two**, a systematic analysis of 195 countries is presented that explores the national public health infrastructure currently in place for the detection of new diseases. We provide a global overview of the evidence of novel disease considerations in national public health laws, notifiable disease lists, health professional guidelines and laboratory guidelines.

In **Chapter Three**, we build upon the findings in Chapter Two by exploring the implications of the different detection thresholds for reporting novel disease outbreaks used by countries. We present a SARS-CoV-2 model and determine the implications of different detection thresholds for time-to-detection and size at outbreak discovery. We expand beyond our

SARS-CoV-2 results by examining diseases with other transmission dynamics using a two-way sensitivity analysis. Potential improvements in surveillance are explored to determine the amount of population-level sampling required to improve upon current detection timeframes for novel disease emergence.

In **Chapter Four**, case detection in the early stages of the COVID-19 pandemic is explored in a community population at a time when there was great uncertainty about the total number of infections and the extent of asymptomatic transmission. Serial cross-sectional prevalence estimates of SARS-CoV-2 infection are presented and four diagnostic methods (RT-PCR, mNGS, serology and the symptom-based case definition) are compared.

In **Chapter Five**, the key research findings of this thesis are summarised and interpreted in the context of current and potential future detection methods for novel disease surveillance. The importance of pathogen-agnostic surveillance capabilities is highlighted and further research directions are proposed.

2

National Surveillance for Novel Diseases: A Systematic Analysis of 195 Countries

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Section 2.1 Abstract

Pandemics caused by novel pathogens produce devastating global effects. Early detection is critical, and the International Health Regulations (2005) (IHR[2005]) require countries to report outbreaks of unknown causes. However, the global extent of novel disease surveillance is unclear. Establishing baseline information is vital for guiding new global preparedness initiatives like the Pandemic Treaty. This Chapter presents a study which aimed to determine the number of countries that consider novel diseases in their national public health infrastructure.

We examined 195 IHR(2005) countries for publicly available evidence of novel disease consideration. This was completed by systematically compiling country-specific information from existing datasets and official government resources. The main outcome was evidence of novel disease consideration in one or more categories: national public health laws, notifiable disease lists, health professional guidelines, and laboratory guidelines. This result was compared using univariate logistic regression against existing indicators, including World Health Organisation (WHO) region, World Bank income strata, health expenditure per capita, total outbreaks per country recorded by the WHO between 1996 – 2022, and national experience with at least one case of the following novel pathogens: SARS-CoV-1, MERS or Nipah virus.

Overall, only 73/195 countries (37.4%) were found to have evidence of novel disease consideration. Compared with the WHO European region, novel disease consideration was significantly less likely in Africa (odds ratio [OR] 0.16 [95% CI 0.061 – 0.40]; *p*-value 0.00014) and the Eastern Mediterranean (0.13 [0.029 – 0.45]; *p*-value 0.0031). Novel disease consideration was less likely in low-income countries (0.16 [0.043 – 0.49]; vs high income;

p-value 0.0025) and was positively associated with increased health expenditure per capita (1.29 [1.09 – 1.56]; per $1000 USD increase; *p*-value 0.0057). A country's total outbreak number was not associated with evidence of novel disease consideration (1.03 [0.99 – 1.07]; *p*-value 0.11), but experience with one or more cases of SARS-CoV-1, MERS or Nipah virus predicted consideration (3.89 [1.94 – 8.04]; *p*-value 0.00017).

A minority of countries have public evidence of novel disease consideration, and significant geographical heterogeneity exists in where this is located. National experience with at least one of three newly emerged pathogens, but not the total outbreak number, predicted novel disease consideration. Countries can act now to improve preparedness and ensure global initiatives like the Pandemic Treaty address novel disease surveillance.

Section 2.2 Research in Context

2.2.1 Evidence Before this Research

We searched Embase, Medline and Global Health via the Ovid online platform for studies published between January 1, 2007 and December 31, 2022, reporting on novel disease surveillance. The start date reflects the year the IHR(2005) entered into force. No language restrictions were applied. The search terms used were: ((("novel disease" or "new disease" or "unknown disease" or "unexplained disease" or "disease of unknown cause" or "disease of unknown aetiology" or "disease of unknown etiology" or "novel pathogen" or "new pathogen" or "unknown pathogen" or "unidentified pathogen") and ("public health" or "health security" or "disease surveillance")).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, cw, bt, nm, ox, px, rx, ui, sy, ux, mx]. This returned 1082 results. Titles and abstracts were screened, with 48 papers, reviews, and conference abstracts retained. Our literature search

demonstrated that novel disease surveillance is acknowledged as a critical public health problem, with increased interest in light of the COVID-19 pandemic. Surveillance for new diseases has been examined at a country level, but we found no studies which assessed the global landscape. “Disease X”, representing any currently unknown pathogen capable of causing a serious international outbreak, has been viewed by the WHO as a priority for improved surveillance. Addressing laboratory preparedness for novel diseases is an important but ongoing challenge, and unbiased tools such as metagenomic next-generation sequencing (mNGS) have been proposed to help identify new pathogens.

2.2.2 Added Value of this Research

This research, to the best of our knowledge, is the first to systematically analyse evidence of novel disease surveillance on a global scale. We present findings on 195 IHR(2005) countries that were examined for evidence of novel disease consideration in their national public health laws, notifiable disease lists, health professional guidelines, and laboratory guidelines. Predictors of novel disease consideration are assessed, and we interpret our results in light of recent advances in mNGS and the potentially forthcoming Pandemic Treaty.

2.2.3 Implications of all the Available Evidence

Only a minority of countries have evidence publicly available that they consider novel diseases in their national public health infrastructure. Novel disease consideration varied significantly by WHO region and national income level. Laboratory preparedness has been identified in the literature as a crucial issue; however, laboratory guidelines were the least common source of novel pathogen considerations found in our research. National governments and the Pandemic Treaty, if adopted, could help safeguard against future novel

pathogen pandemics by addressing global disparities and explicitly including novel diseases in public health surveillance.

Section 2.3 Introduction

Pandemics caused by novel pathogens, such as SARS-CoV-2, can result in unparalleled consequences for health, economies and societies.[104] A primary objective of infectious disease surveillance is early outbreak detection, as this enables the timely implementation of public health interventions that reduce transmission and prevent pandemics. In the wake of COVID-19, a crucial question for all national public health systems is whether their surveillance infrastructure is capable of rapidly detecting future, currently unknown diseases.

Detecting novel diseases with pandemic potential is in the interest of all countries. Reporting to the WHO is also mandatory under the legally-binding International Health Regulations (2005) (IHR[2005]), which require its 196 signatory Member States to notify "any event of potential international public health concern, including those of unknown causes."[43] However, reporting requires detection, and neither the IHR(2005) nor its monitoring framework provides guidance to countries on how to identify such outbreaks, leaving the responsibility of developing surveillance tools to countries.[43,105,106]

New diseases pose unique challenges to public health surveillance. For example, when novel pathogens first emerge, their symptom profile is unknown and their causative agent is uncharacterised. Commonly used diagnostic tests, such as RT-PCR, are limited to known diseases, and laboratories are not always equipped with hypothesis-free tools such as metagenomic next-generation sequencing (mNGS) to identify new pathogens.[59,63]

The previous emergence of novel viruses offers some insights into some of the challenges faced in their detection. When Nipah virus first emerged in humans in Malaysia in 1998, it was initially mistaken for Japanese Encephalitis despite having an unusual clinical presentation; a novel agent was not recognised until cases appeared the following year in Singapore and a coordinated investigation was undertaken.[84] When Severe Acute Respiratory Syndrome (SARS) caused by the SARS-CoV-1 virus was first recognised in 2003, infections were retrospectively identified from months prior.[107] The novel Middle East Respiratory Syndrome Coronavirus (MERS) was only discovered after repeated negative tests for known diseases in a fatal case were followed up by a physician who organised sample sharing between Saudi Arabia and the Netherlands for aetiological characterisation.[108]

All of these emerging disease outbreaks involved some level of initial delay in novel virus discovery and required above-and-beyond actions by health professionals. As is evident from those events, there can be multiple barriers to the timely reporting of a new disease to public health authorities. Novel infections may mimic known diseases, and not every clinical presentation receives a definitive diagnosis.[109,110] Clinicians may not suspect, or could be hesitant to notify, if clear reporting guidance is unavailable or novel diseases are not included on the nationally notifiable disease list.

Despite these barriers, there is some evidence that countries have taken steps to reduce their risk from novel disease outbreaks and meet IHR(2005) requirements. Every year, 196 IHR(2005) countries are asked to self-report on 15 capacities comprised of 35 indicators through the electronic State Party Self-Assessment Annual Reporting (SPAR) tool.[111] Following 2021 revisions to the SPAR, countries are now for the first time asked annually whether their laboratories or an international collaborator can perform “identification of

unknown and high consequence pathogens", with full text of the SPAR indicator available in Appendix A. In 2021, 52/184 countries (28.3%) that completed a report stated they had access to this laboratory capability. By 2022, this had only slightly increased to 54/186 (29.0%) of reporting IHR(2005) countries. This low number of countries with access to laboratory testing capacity for unknown disease threats is a major concern for global public health given the potential consequences of novel pathogen outbreaks.

The 2021 Global Health Security Index (GHSI) has also examined a range of pandemic preparedness indicators through 171 questions.[48] One of these asks whether the country has a national plan for laboratories in public health emergencies that includes novel pathogen testing (full text of question available in Appendix A). In 2021, 0/195 (0%) met all the criteria. Approaching the problem from another angle, event-based surveillance can use various data sources to rapidly detect new outbreaks, including those caused by novel pathogens.[112] However, the 2021 GHSI found only 89/195 (45.6%) nations operate event-based surveillance, with just 18/195 (9.2%) conducting daily data analysis.[48]

The SPAR and GHSI provide information on laboratory capabilities and event-based surveillance through these indicators but do not fully address whether countries consider novel diseases in their public health surveillance, for example through national law, disease notification lists or specific guidelines. There has been some previous research outlining novel disease detection in specific countries such as Bangladesh,[52] or comparing how the disease surveillance systems of China and the Netherlands mandate notification of unknown diseases.[113] However, no study to date has systematically analysed to what extent novel diseases are considered globally by national public health systems.

In this Chapter, we examine IHR(2005) countries for publicly available evidence of novel disease consideration. Such information primarily exists in government publications, national guidelines, and public health laws. Previous studies have highlighted the importance of the use of this literature to answer public health questions that would otherwise remain unexamined.[114–116] Through systematic analysis of available material, we assessed countries for evidence of novel disease consideration and explored factors associated with their inclusion.

Section 2.4 Methodology

A total of 195 of 196 countries that signed the IHR(2005) were included in this research. The Holy See (Vatican), while being an IHR(2005) State Party and sovereign legal separate entity, was excluded because the Vatican Constitution states that matters concerning infectious diseases are delegated to Italian law.[117] Data collection took place between July and December 2022. All sources were re-examined in January 2023 for any new publications, with updates made to the dataset if required. A data date cut-off of 31 December 2022 was chosen to standardise comparison between countries. As the primary focus of this research was to examine evidence of consideration of novel diseases at the national level, resources published by a regional or international public health organisation, or at the subnational level, were excluded. A summary of the definitions and search strategy is included below, with full information available in Appendices A and B.

2.4.1 Definitions for Novel Disease Consideration

Novel disease consideration was defined as the inclusion of any written material designated at the national level on novel diseases in one or more of the following resources: public

health laws, notifiable disease lists, health professional guidelines, or laboratory guidelines. Public health laws included national legislation, decrees, acts, and orders related to public health, epidemics, infectious diseases or their national surveillance systems. Nationally notifiable disease lists consisted of all communicable diseases under mandatory reporting requirements. Health professional guidelines included published government documentation detailing the national disease surveillance system or reporting guidance directed at health professionals in direct contact with ill persons, including doctors and community health workers. Laboratory guidelines included any government-published guidance on requirements for laboratories to investigate and report infectious diseases under national surveillance.

For both health professional and laboratory guidelines, evidence of the inclusion of novel diseases was classified under four categories: "written guidance", "form/protocol", "written guidance and form/protocol", and "only mentioned." Written guidance included a dedicated section in the document on novel diseases. For health professional guidelines, it was recorded whether there was a specific form available for reporting novel diseases to public health authorities. For laboratory guidelines, information was collected on whether there was a protocol to investigate suspected novel pathogens. For both health professional and laboratory guidelines, if novel diseases were mentioned in a single sentence without further instructions or details provided, this was classified as "only mentioned." The motivation for including this category was to be inclusive of any public evidence of novel disease consideration, given that internal preparedness activities were beyond the scope of this study to measure. National disease surveillance data were classified as publicly available notifiable infectious disease reports which included more than one disease or disease group, published at the national level.

2.4.2 Search Strategy and Selection Criteria

To determine whether countries had evidence of novel disease consideration available in publicly accessible resources, a search strategy was developed based on previously described methods and guidelines adapted to analysing government sources.[48,114–116,118] A summary of the search strategy is shown in Figure 2.1, with full details in Appendices A and B. First, for each country, potential resource locations, including relevant government websites and legal repositories, were identified by examining existing databases and resources, such as each World Health Organisation (WHO) Joint External Evaluation report and the 2021 GHSI.[48] Resources in four categories were then screened for evidence of novel disease consideration for every included country: national public health laws, nationally notifiable disease lists, health professional guidelines and laboratory guidelines. In addition, data were collected from the national resources identified to gather a primary comparative picture of each country's infectious disease surveillance system, including the number of diseases under surveillance, how frequently surveillance data are analysed, and the latest published national disease surveillance reports, as available and accessible.

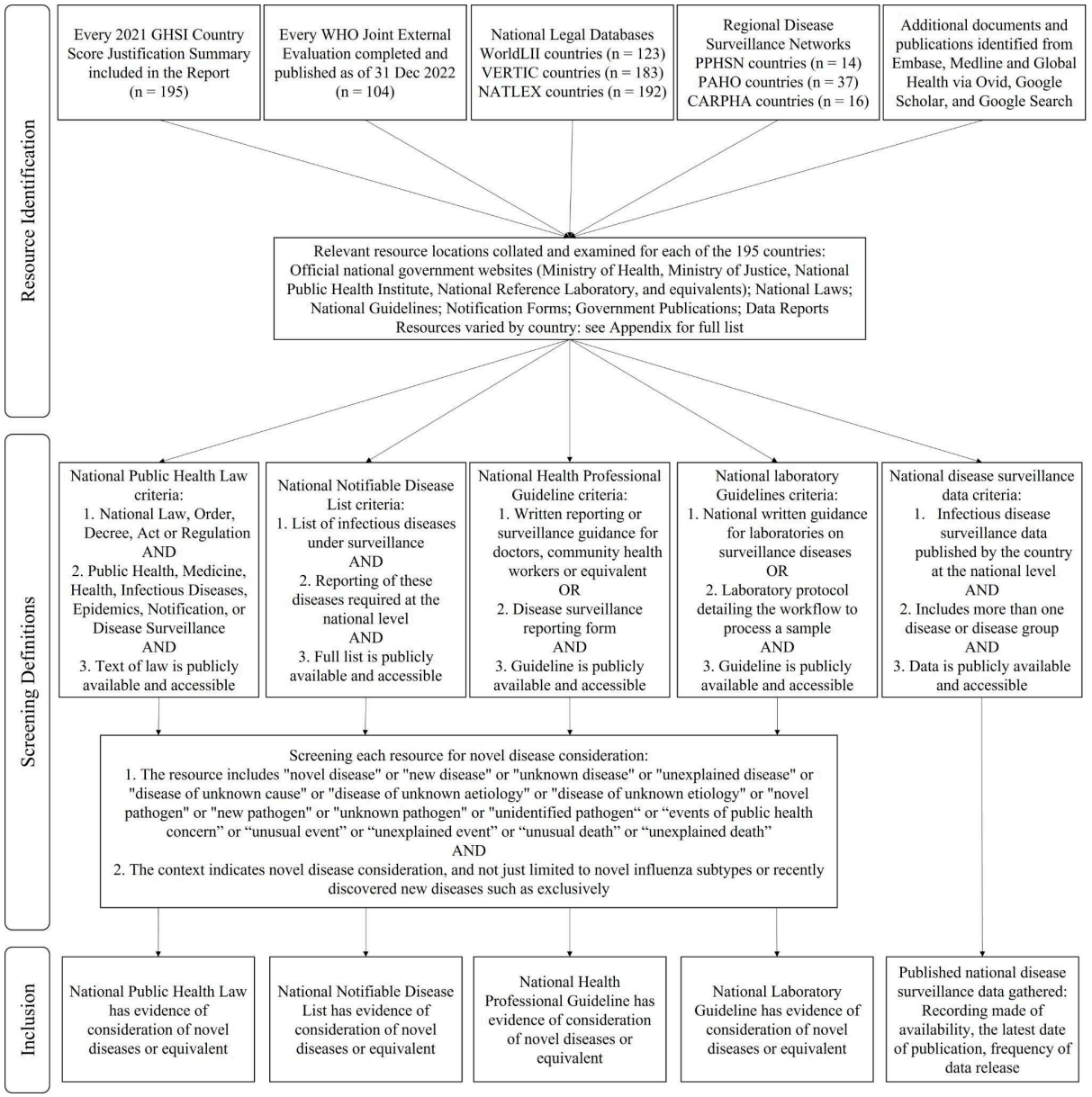


Figure 2.1 Summary of the search strategy used in the data collection process for the study, including identification of relevant resources for the 195 included countries and screening criteria for novel disease consideration. The resources identified varied by country and the full search strategy, including search terms and resources, can be found in Appendices A and B. GHSI: Global Health Security Index; WHO: World Health Organisation; WorldLII: World Legal Information Institute; VERTIC: Verification Research, Training and Information Centre; NATLEX: Database of national legislation; PPHSN: Pacific Public Health Surveillance Network; PAHO: an American Health Organization; CARPHA: Caribbean Public Health Agency.

2.4.3 Statistical Analysis

The main outcome investigated was "any evidence of novel disease consideration" in one or more of the four resources (national public health law, notifiable disease list, health professional guidelines, or laboratory guidelines) examined for each included country. This

outcome was compared against a range of other metrics and indicators collected for this study to determine predictors of novel disease consideration. This included the 2021 GHSI,[48] the 2021 SPAR,[111] and all WHO Joint External Evaluations completed and published by 31 December 2022. In addition, comparisons were made against World Bank income strata based on 2021 reported Gross National Income, and 2019 data on health expenditure per capita.[119,120] The outcome was also compared against the total number of outbreaks a country had experienced between January 1996 to March 2022, using a published global outbreak dataset with 2059 WHO records for 194 of the included countries.[121] In addition, evidence of novel disease consideration was examined in countries that had experienced one or more cases of one of the following newly emerged diseases: MERS, SARS-CoV-1, or Nipah virus. These inclusions were to test whether novel disease consideration was associated with greater numbers of reported outbreaks or national experience with a novel pathogen, excluding SARS-CoV-2, given its universal reach.

The Welch two-sample t-test was used to compare the value of continuous quantities such as the 2021 GHSI score between countries with and without evidence of novel disease consideration. A chi-squared (χ^2) test of independence was used to compare the proportions of countries with and without evidence of novel disease considerations across categorical variables, with the degrees of freedom (*df*) and sample size (N) reported. Univariate logistic regression was conducted to determine the relationship between individual predictor variables and the binary outcome of whether countries had any consideration of novel diseases or not.

This method generates the odds ratio (OR) to quantify the strength and direction of the relationship between the predictor and the outcome, with 95% confidence intervals (CIs) reported. A p-value < 0.05 was deemed significant.* All data were analysed in RStudio with R Version 4.2.2.[122] Ethics approval was not required, as this research consisted of national-level public data collection and did not involve human research subjects.

Section 2.5 Results

Out of the 195 countries included, 73 (37.4%) had any evidence of novel disease consideration in the four resource types investigated (national public health law, notifiable disease list, health professional guidelines, and laboratory guidelines), with the global distribution shown in Figure 2.2. Evidence of any novel disease consideration varied significantly by WHO region, from 8/47 (17.0%) of countries in the African Region to 30/54 (55.6%) in the European Region, with χ^2 (df = 5, N = 195) = 23.44, p-value = 0.00028. There was a strong association between evidence of novel disease consideration and World Bank income strata, ranging from 4/27 (14.8%) countries with low income to 30/58 (51.7%) countries with high income, with χ^2 (df = 3, N = 192) = 14.38, p-value = 0.0024. Overall, only 8/195 (4.1%) countries had evidence of novel disease consideration in all four resource locations. None of these countries had both health professional and laboratory guidance that included written guidelines, forms and protocols.

* A p-value < 0.05 is the conventional threshold for determining statistical significance in many scientific disciplines, including public health. While this value is broadly recognised and ubiquitously used, it is potentially too liberal given it incurs an up to 5% chance of incorrectly rejecting the null hypothesis (Type I error) leading to false positive results. In addition, it can put too much emphasis on an artificially binary view of significant vs. non-significant rather than interpreting results within their full context. In this Chapter, to foster transparency and enable nuanced interpretation, all individual p-values ≥ 0.0001 are reported throughout, allowing the reader to judge for themselves the degree of statistical evidence against the null hypothesis.

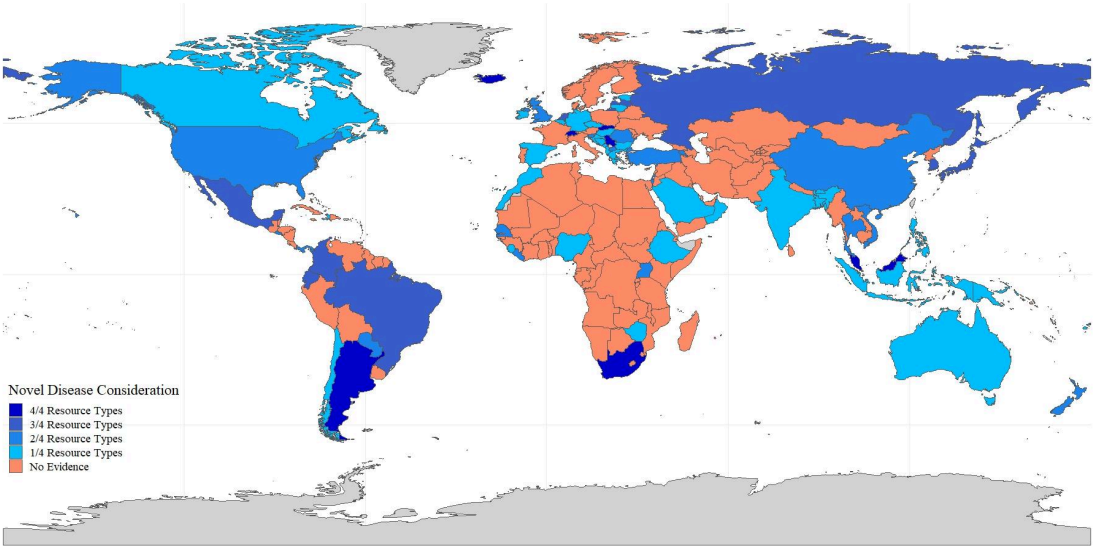


Figure 2.2 Global map depicting evidence of novel disease inclusion in any of the four resource types (public health law, national notifiable disease list, health professional guidelines, and laboratory guidelines) for 195 countries. Evidence of novel disease consideration in one or more of the four resources is shown in blue, while countries with no evidence are depicted in red.

Overall, 38/195 countries (19.5%) had evidence of reporting requirements for novel diseases included in national public health law. As shown in Table 2.1, the proportion of countries with such legal instruments varied by World Bank income strata, from 1/27 (3.7%) low-income countries to 17/53 (32.1%) upper-middle-income countries. There was a significant and positive association between evidence of novel disease consideration in national public health law and income level, with χ^2 (df = 3, N = 192) = 14.56, p-value = 0.0022. Countries with novel diseases included in national public health law also varied significantly by WHO Region, from 1/21 (4.8%) in the Eastern Mediterranean to 22/54 (40.7%) in the European Region, with χ^2 (df = 5, N = 195) = 26.51, p-value = <0.0001.

Table 2.1 Novel disease consideration in four national resource types (public health law, nationally notifiable disease list, health professional guidelines, and laboratory guidelines) organised by World Bank income strata and WHO region for included countries. WHO: World Health Organisation; AFRO: African Region; AMRO: Region of the Americas; EMRO: Eastern Mediterranean Region; EURO: European Region; SEARO: South-East Asian Region; WPRO: Western Pacific Region.

Category	Number of Countries	Novel Disease Consideration				
		National Public Health Law	Nationally Notifiable Disease List	Health Professional Guidelines	Laboratory Guidelines	*One or more of the four resources*
	n	n (%)	n (%)	n (%)	n (%)	*n (%)*
World Bank Income Strata*						
High	58	15 (25.9)	9 (15.5)	20 (34.5)	12 (20.7)	*30 (51.7)*
Upper Middle	53	17 (32.1)	14 (26.4)	15 (28.3)	9 (17.0)	*24 (45.3)*
Lower Middle	54	5 (9.3)	4 (7.4)	9 (16.7)	0 (0)	*15 (27.8)*
Low	27	1 (3.7)	2 (7.4)	3 (11.1)	0 (0)	*4 (14.8)*
WHO Region						
AFRO	47	3 (6.4)	4 (8.5)	6 (12.8)	1 (2.1)	*8 (17.0)*
AMRO	35	4 (11.4)	9 (25.7)	10 (28.6)	6 (17.1)	*13 (37.1)*
EMRO	21	1 (4.8)	1 (4.8)	1 (4.8)	0 (0)	*3 (14.3)*
EURO	54	22 (40.7)	12 (22.2)	15 (27.8)	9 (16.7)	*30 (55.6)*
SEARO	11	1 (9.1)	0 (0)	5 (45.5)	1 (9.1)	*6 (54.5)*
WPRO	27	7 (25.9)	3 (11.1)	10 (37.0)	4 (14.8)	*13 (48.1)*
Global	195	38 (19.5)	29 (14.9)	47 (24.1)	21 (10.8)	*73 (37.4)*

* Data based on 2021 reported Gross National Income;[119] data unavailable for Venezuela, Cook Islands and Niue

Only 29/195 countries (14.9%) had evidence that they included novel diseases in their nationally notifiable disease list. The inclusion of novel diseases on the list was associated with the country's income strata, with χ^2 (df = 3, N = 192) = 9.04, p-value = 0.029, but was not significantly associated with WHO region, with χ^2 (df = 5, N = 195) = 10.98, p-value = 0.052.

A total of 47 countries (24.1%) had evidence of health professional guidance for novel diseases. This proportion varied significantly by WHO region, with χ^2 (df = 5, N = 195) = 13.59, p-value = 0.018, and by World Bank income strata, with χ^2 (df = 3, N = 192) = 7.95, p-value = 0.047. From the subset of 47 countries with evidence of novel disease guidance, 20

(42.6%) had written health professional guidelines, two (4.3%) had a reporting form, ten (21.3%) had both written guidelines and a form, and 15 (31.9%) only mentioned novel diseases in publicly available resources.

Out of the 195 countries, only 21 (10.8%) had evidence of laboratory guidance for novel diseases. The proportion of countries with evidence in laboratory guidance varied by income strata, with χ^2 (df = 3, N = 192) = 17.60, p-value = 0.00053, but was not significantly associated with WHO region, with χ^2 (df = 5, N = 195) = 10.11, p-value = 0.072. From the subset of 21 countries with evidence of laboratory novel disease guidance, nine (42.9%) had written laboratory guidelines, one (4.8%) had a laboratory protocol, one (4.8%) had both written guidelines and a protocol, and ten (47.6%) only mentioned novel diseases in published resources.

Evidence of novel disease consideration varied by overall 2021 GHSI and SPAR scores and specific indicators, as shown in Figure 2.3. The average 2021 GHSI score was significantly greater for countries with evidence of novel disease consideration compared with countries without evidence (48.6 vs 33.1, Welch two-sample t-test p-value <0.0001). A similar significant difference was found for the average 2021 SPAR score when comparing countries with evidence of novel disease consideration vs no evidence (73.2 vs 58.6, p-value <0.0001). Countries with novel disease consideration outperformed countries without across all capacity categories in both the GHSI and SPAR in 2021.

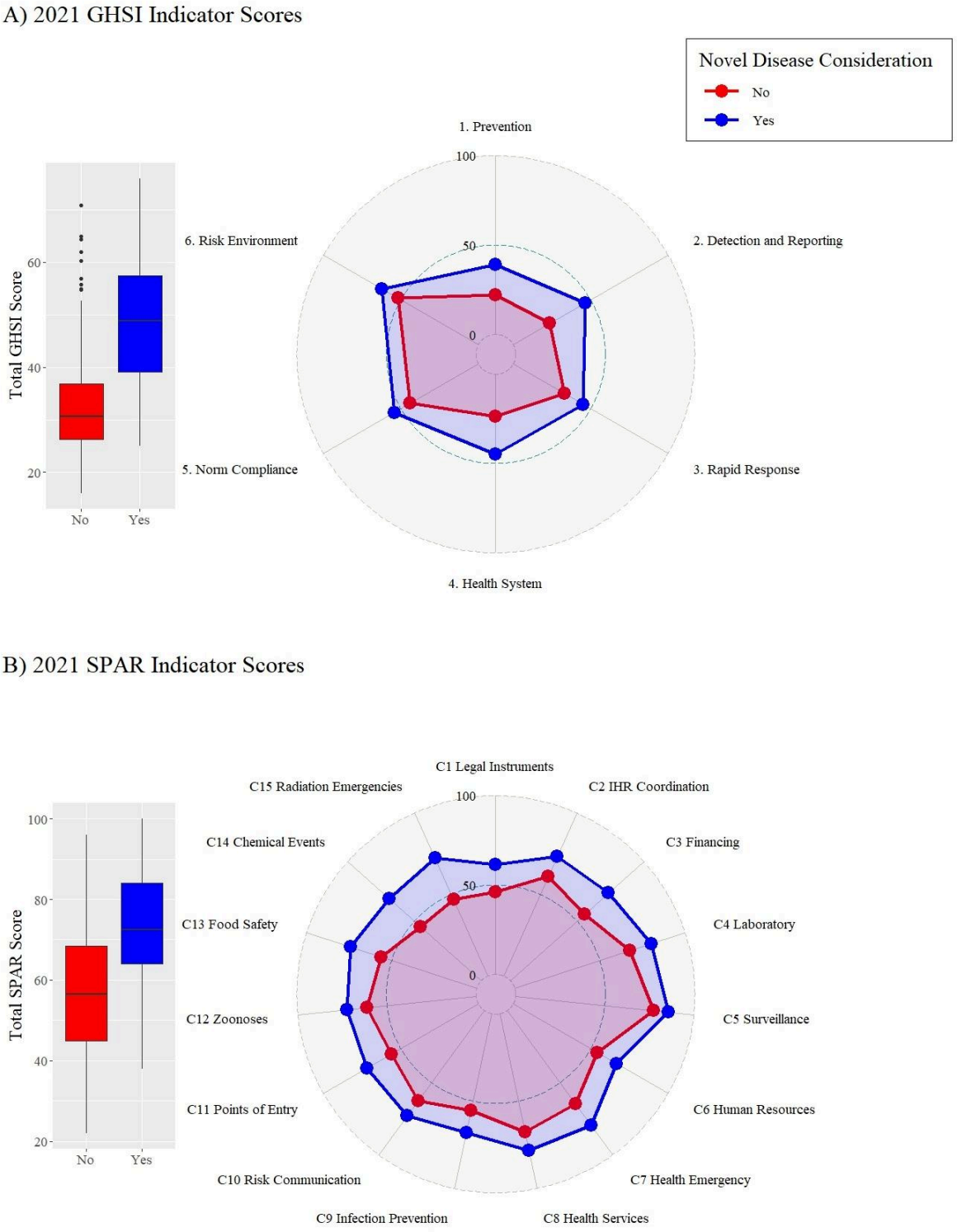


Figure 2.3 Novel disease consideration in any of the four examined resource types investigated (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines) compared against existing metrics: A) 2021 GHSI Score for 195 countries with data available, scored from 0 to 100; B) 2021 SPAR score for 184 countries with data available, with score range possible from 20 to 100. For both the 2021 GHSI and SPAR, higher scores indicated that more criteria had been met for the given capacity examined. Novel disease consideration in one or more resource types in blue with no evidence of consideration in red. GHSI: Global Health Security Index; SPAR: State Party Self-Assessment Annual Reporting; WHO: World Health Organisation; IHR: International Health Regulations.

Univariate logistic regression was used to identify factors statistically associated with evidence of novel disease consideration in any of the four resource types investigated (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines). The results of this are shown in Table 2.2. When compared with countries with high income as classified by the World Bank, countries in the lower-middle and low-income strata were significantly less likely to have novel disease consideration in one or more of the examined resources, with an OR (95% CI; *p*-value) of 0.36 (0.16 – 0.78; 0.011) and 0.16 (0.043 – 0.49; 0.0025), respectively. Compared with the WHO European Region, countries in the African and Eastern Mediterranean Regions were significantly less likely to have any evidence of novel disease consideration, with an OR (95% CI; *p*-value) of 0.16 (0.061 – 0.40; 0.00014) and 0.13 (0.029 – 0.45; 0.0031), respectively.

Table 2.2 Predictors of any evidence of novel disease consideration in at least one of four resources (public health law, national notifiable disease list, health professional guidelines, and laboratory guidelines) compared with no evidence using univariate logistic regression. WHO: World Health Organisation; AFRO: African Region; AMRO: Region of the Americas; EMRO: Eastern Mediterranean Region; EURO: European Region; SEARO: South-East Asian Region; WPRO: Western Pacific Region; USD: United States Dollar; SARS-CoV-1: Severe acute respiratory syndrome coronavirus 1; MERS: Middle East Respiratory Syndrome; GHSI: Global Health Security Index; SPAR: State Party Self-Assessment Annual Reporting; OR: Odds Ratio; CI: Confidence Interval.

Predictor	Novel Disease Consideration		
	n/N (%)	OR (95% CI)	*p*-value
World Bank Income Strata*			
High	30/58 (51.7)	Referent	-
Upper Middle	24/53 (45.3)	0.77 (0.36 – 1.63)	0.50
Lower Middle	15/54 (27.8)	0.36 (0.16 – 0.78)	0.011
Low	4/27 (14.8)	0.16 (0.043 – 0.49)	0.0025
WHO Region			
EURO	30/54 (55.6)	Referent	-
AFRO	8/47 (17.0)	0.16 (0.061 – 0.40)	0.00014
AMRO	13/35 (37.1)	0.47 (0.19 – 1.12)	0.092
EMRO	3/21 (14.3)	0.13 (0.029 – 0.45)	0.0031
SEARO	6/11 (54.5)	0.96 (0.26 – 3.70)	0.95
WPRO	13/27 (48.1)	0.74 (0.29 – 1.88)	0.53
Health expenditure, $1000 USD per capita[†]	71/186 (38.2)	1.29 (1.09 – 1.56)	0.0057
National Disease Surveillance Data Available[‡]			
Yes	64/122 (52.5)	7.85 (3.74 – 18.18)	<0.0001
No	9/73 (12.3)	Referent	-
Completed and Published WHO Joint External Evaluation			
Yes	37/104 (35.6)	0.84 (0.47 – 1.51)	0.57
No	36/91 (39.6)	Referent	-
WHO Joint External Evaluation Score	37/104 (35.6)	1.07 (1.04 – 1.11)	<0.0001
Total Recorded Outbreaks[§]	73/194 (37.6)	1.03 (0.99 – 1.07)	0.11
One or more cases of SARS-CoV-1, MERS, or Nipah Virus			
Yes	27/43 (62.8)	3.89 (1.94 – 8.04)	0.00017
No	46/152 (30.3)	Referent	-
2021 GHSI Score	73/195 (37.4)	1.11 (1.08 – 1.15)	<0.0001
2021 SPAR Score[‖]	70/184 (38.0)	1.05 (1.03 – 1.08)	<0.0001

***** Data from the World Bank based on 2021 reported Gross National Income;[119] unavailable for Venezuela, Cook Islands and Niue

[†] Data from the World Bank based on the latest national health expenditure estimates from 2019;[120] unavailable for Albania, Cook Islands, Libya, Liechtenstein, Niue, North Korea, Somalia, Syria and Yemen

[‡] Publicly published national infectious disease surveillance data available as of 31 December 2022

[§] Data from a dataset covering the period January 1996 – March 2022;[121] unavailable for Turkmenistan

[‖] Data based on country SPAR self-reports submitted as of 31 December 2022;[111] unavailable for Andorra, Barbados, Brunei, Grenada, Israel, Marshall Islands, Nauru, Niue, Papua New Guinea, Trinidad and Tobago, and Turkey

For every $1000 USD per capita increase in health expenditure, the odds (95% CI) of novel disease consideration increased by 1.29 (1.09 – 1.56), with a *p*-value of 0.0057. Having completed and published a WHO Joint External Evaluation was not associated with evidence of novel disease consideration, with an OR (95% CI; *p*-value) 0.84 (0.47 – 1.51; 0.57), but each point increase in the score (conditional on having completed and published a Joint External Evaluation) had an increased OR (95% CI; *p*-value) of 1.07 (1.04 – 1.11; <0.0001). This may be explained in part because of significant regional variation in WHO Joint External Evaluation completion, from 2/35 (5.7%) countries in the Region of the Americas having completed and published an Evaluation to 45/47 (95.7%) in the African Region, with a χ^2 (df = 5, N = 195) = 84.37, *p*-value <0.0001.

A country experiencing more recorded outbreaks between 1996 – 2022 was not associated with a higher chance of novel disease consideration, with an OR (95% CI; *p*-value) of 1.03 (0.99 – 1.07; 0.11). However, national experience with at least one case of SARS-CoV-1, MERS or Nipah Virus did predict novel disease consideration, with an OR (95% CI; *p*-value) of 3.89 (1.94 – 8.04; 0.00017). Countries with national disease surveillance data publicly available were significantly more likely to have evidence of novel disease consideration, with an OR (95% CI; *p*-value) of 7.85 (3.74 – 18.18; <0.0001). Similarly, countries which scored higher on the 2021 GHSI and SPAR were more likely to have evidence of novel disease consideration, with each point increase in total score associated with an OR (95% CI; *p*-value) of 1.11 (1.08 – 1.15; <0.0001) and 1.05 (1.03 – 1.08; <0.0001), respectively.

Section 2.6 Discussion

Fewer than two-fifths of countries globally have publicly available evidence of novel disease consideration in their national health professional and laboratory guidelines, public health laws or notifiable disease lists. Only 8/195 (4.1%) countries had evidence in all four resource locations, and none of these included instructions, forms and protocols for both health professionals and laboratories. Health professional guidelines were the most common source for novel disease inclusion, but still only present in 47/195 (24.1%) countries.

Laboratory guidelines were the least common source, with only 21/195 countries (10.8%) at least mentioning testing for novel pathogens. Previous work has highlighted the difficulties laboratory staff face when a new disease emerges and there is an absence of relevant protocols for pathogen identification and reporting.[123,124] This challenge, coupled with the comparative rarity of accessible laboratory guidelines that our work has demonstrated, underscores the crucial need for the creation and dissemination of guidance for the laboratory detection of novel pathogens. Furthermore, expanding access to unbiased tools like mNGS could support the identification of new pathogens through agnostic sequencing.[62,68]

Developing mNGS surveillance guidelines may be a part of the solution. It has been argued that there is a strong case for mNGS expansion for early outbreak detection in all nations, even in low- and middle-income countries, given the benefits and opportunity afforded by the infrastructure developed for SARS-CoV-2 genomic surveillance.[125] The development of protocols for novel pathogen surveillance is particularly important in low and lower-middle income countries, as we found that evidence of novel disease consideration was significantly

less likely in those regions. This is concerning, given these countries are disproportionately at the highest risk of zoonotic spillover as the world's climate continues to change this century.[9]

In this study, the odds of novel disease consideration increased with health expenditure per capita and was significantly more likely in countries which publicly publish notifiable disease surveillance data. These results indicate that, as might be expected, countries with more public health investment are more likely to have considered novel diseases. This is consistent with the finding that surveillance for new diseases is associated with higher total 2021 SPAR and GHSI scores and each individual capacity of these metrics.

Interestingly, novel disease consideration was not associated with the total number of reported outbreaks that a country has experienced. However, countries with at least one case of SARS-CoV-1, MERS or Nipah virus are significantly more likely to have evidence of novel disease consideration. This could be in part explained by the geographical disparity in outbreak risk.[126] It is also possible that the experience of having to respond to a novel disease threat, but not to known pathogen outbreaks, encourages the inclusion of novel diseases in national surveillance infrastructure.

Public health law is an important component of this infrastructure and is required for pandemic policy implementation, but research has demonstrated it is underutilised for novel disease preparedness.[127] In our research, we found only a minority of countries with novel disease considerations included in their national public health laws (38/195, 19.5%), and even fewer with them on their notifiable disease list (29/195, 14.9%). Underreporting of notifiable conditions is already known to be a pervasive challenge in public health.[54] However, if surveillance for novel diseases is not mandated in law or included on the notifiable disease

list, we are effectively choosing to rely on ad hoc actions of health professionals in the initial stages of a novel pathogen outbreak. The lack of guidance and notification tools for frontline workers has been found to impede reporting to public health authorities.[106] This should be a concern to all nations, as delaying the timely recognition of the next "Disease X" could hinder control efforts and make a global pandemic more likely.

Globally, there is a need for novel pathogen detection standards. Countries may have developed approaches they do not disclose publicly, but to facilitate the formation of a gold standard approach and accurately evaluate global preparedness, best practices should be shared. In the aftermath of the COVID-19 pandemic, a Pandemic Treaty that complements the IHR(2005) could help deliver this, preparing nations for future disease outbreaks.[128] It has been argued that such a legally-binding Treaty, tabled for adoption in May 2024 by WHO Member States, needs to address building surveillance capacity and genomic sequencing capabilities as key components.[129] As demonstrated by the research findings presented in this Chapter, there is currently insufficient consideration given to novel diseases in existing public health surveillance infrastructure. Surveillance for "Disease X" and universalising laboratory access to pathogen-agnostic tools like mNGS are important for the Pandemic Treaty if it is to safeguard against future, currently unknown threats. Existing resources mean that nothing has to be reinvented; the current strategies of global neighbours can be adapted to national contexts and implemented.

2.6.1 Limitations and Future Directions

Like any research of this nature, this study has limitations. The resources examined were restricted to those that are publicly available and accessible and do not necessarily reflect internal preparedness for novel disease threats within national public health systems.

Countries may have changed practice in light of COVID-19, but not yet updated public information sources due to the strain on public health workforces. We encourage transparent and accessible sharing of current practices so that nations can draw on the insights of each other's public health professionals. Our research was designed to detect associations, and determining any causal links between our predictors and novel disease consideration could be the aim of future work. Furthermore, other forms of surveillance system comparison were outside the scope of this Chapter, but our comprehensive resource collection available for use (see Appendix B for full details) could be used to compare public health laws, national notifiable diseases and surveillance data reporting practices between countries.

2.6.2 Conclusion

The COVID-19 pandemic has highlighted the need to prepare for threats from novel pathogens. However, our research has shown that only a minority of countries have evidence of any consideration of new diseases in their public health infrastructure. Pathogens do not respect international borders and robust strategies for rapid novel outbreak detection in all countries are essential to safeguard global public health. The Pandemic Treaty negotiations are a promising development, but public health policymakers worldwide should not wait to strengthen their national surveillance systems. Preparedness for future unknown disease threats can and should be undertaken now.

2.6.3 Data Availability

All data used in this Chapter are available for download through the Open Science Framework platform, along with all code used to generate the results, tables and figures. ❄ The dataset description and resources are included in Appendices A and B. Additional results are available in Appendix C.

3

Modelling Detection and Surveillance for Novel Pathogen Outbreaks

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Section 3.1 Abstract

When novel pathogens first emerge, they can spread undetected in the initial stages of the outbreak. Discovery delays can result in large outbreak sizes upon detection, hindering the public health response. As we demonstrated in Chapter Two, current surveillance for new pathogen outbreaks differs worldwide, with most countries having no publicly available evidence of novel disease consideration in their national public health infrastructure. In countries with established requirements, discovery relies primarily upon symptomatic cases presenting to a health service. The national detection thresholds used by countries (Appendix B) varied between one and five hospital cases and up to two deaths before notification is mandated. It is currently unknown what impact these different detection thresholds between countries could have on the potential time-to-discovery or total outbreak size after a novel pathogen emerges. Looking beyond current systems used to detect novel disease outbreaks, there have been calls to incorporate pathogen-agnostic technology such as metagenomic next-generation sequencing (mNGS) into population-level surveillance for early warning systems. However, the amount of testing required to detect a novel disease outbreak using such strategies remains unclear.

In this Chapter, we employ a stochastic model based on SARS-CoV-2 transmission dynamics and simulate 100,000 outbreaks in a population of 100,000 persons commencing with a single exposed individual. We determine, for all large outbreaks resulting in at least one hospitalised case, the day and total outbreak size by which 50% of the outbreaks will be detected, considering the different criteria used by countries. We extend our results through a sensitivity analysis, re-running the model with variations of the parameters to see how the results generalise beyond SARS-CoV-2 transmission dynamics. To investigate new surveillance strategies for early detection, we determine what level of testing coverage through daily random sampling would be required to detect the outbreak prior to the first hospitalisation, or within the first seven, 14 or 28 days of the outbreak commencing.

Our findings reveal that for all SARS-CoV-2 outbreaks that do not go extinct in the early stages, 50% will be detected through the first hospital presentation by 46 days. By this time, the outbreak has already caused 155 total infections. We demonstrate that for variations in the model parameters, such as a disease with a long latent period, even greater time delays to detection are possible. To detect 50% of SARS-CoV-2 outbreaks before the first hospital presentation, the minimum surveillance level required is 0.069% (Interquartile Range: 0.028 - 0.139%) of the total population per day.

Our research highlights the importance of early detection given the potential delays and size at outbreak discovery with existing approaches. The results we present can assist public health authorities in assessing the feasibility of population-level testing and designing surveillance systems that potentially outperform current detection for novel pathogens.

Section 3.2 Introduction

Nearly all outbreaks of international significance in recent years have been due to emerging pathogens.[4] However, when an infectious agent first emerges, it can initially remain unrecognised by public health authorities as it silently spreads through the host population. This is because discovering a new disease generally relies on symptomatic individuals presenting themselves to a health service, which in turn depends on various factors, including the likelihood and rate of developing symptoms severe enough to require medical attention. Traditionally, infectious disease surveillance systems rely upon symptomatic medical presentations with health professionals identifying and reporting cases to public health authorities.[53] However, in various scenarios, such as when a new disease causes asymptomatic or mild illness in a proportion of those infected, the first clusters of cases are not identified if individuals do not seek medical attention. Further delays may be possible given the widespread lack of reporting guidelines for new diseases in most countries as demonstrated in Chapter Two. Without early detection, a new outbreak may grow significantly in size before it comes to the attention of public health authorities.[42] The longer a pathogen transmits unrecognised, the further the epidemic could spread, increasing the risk that containment becomes impossible and a pandemic may result.

There have been various modelling studies demonstrating that early detection is important for outbreak control and significantly reduces the cost of the public health response.[130–132] There has also been significant work on developing outbreak detection algorithms from syndrome case data, although these methods also generally rely on symptomatic individuals presenting to health services.[133–136] This provides an imperative to investigate whether detection through healthcare presentations is timely or whether surveillance through other monitoring strategies

could improve time-to-detection. Despite the public health importance of early outbreak discovery, it has been noted that few modelling studies have examined how to sample a population to achieve detection in the first place.[137] There are some notable exceptions to this, including previous work on epidemic discovery in animals showing that consistent random population sampling can allow for detection at low prevalence levels.[138] Further research has demonstrated methods to determine the incidence and prevalence at first discovery using random sampling, and that frequent testing at regular intervals is important for the early detection of an outbreak.[137,139] However, these strategies have previously only been applied to specific known diseases, and it is unknown how sampling strategies for early detection change given novel pathogens with differing transmission dynamics.

3.2.1 SARS-CoV-2 Detection

The novel coronavirus SARS-CoV-2, responsible for the COVID-19 pandemic, exemplifies the pressing need for early detection of new infectious pathogens. The outbreak was first discovered when four hospitalised cases of pneumonia of unknown aetiology were reported to public health authorities on 30 December 2019 in Wuhan, Hubei Province, China.[96] It is now known that this date did not represent the beginning of the outbreak, with the first known symptom onset occurring on 1 December 2019 and the first hospital admission on 16 December 2019.[98] How long the outbreak had been spreading and its true size at the time of detection has not been established with certainty, although there has been active research in this area. For example, modelling estimates from two studies suggest the index case in Wuhan occurred sometime between early October to mid-November 2019.[140,141]

Various estimates of the true size of the COVID-19 outbreak in the early phase have also been reported in the literature. One early modelling estimate by Imperial College London

considered the international travel volume from Wuhan and the number of confirmed cases outside of China and modelled that there had been 1,723 symptomatic cases (95% confidence interval [CI]: 427-4,471) of SARS-CoV-2 infections in Wuhan with symptom onset by 12 January 2020.[142] At the time, as the report states, there were 41 known cases and two deaths in Wuhan, China and three international cases. This implies that in Wuhan, only 2.4% (0.92 - 9.6%) of symptomatic infections may have been known to public health authorities by this date. The total number of infections, including asymptomatic, would have been even greater, making the proportion of identified cases even smaller. Another study estimated that there had been 469 unreported cases (95% CI: 403–540) from 1 to 15 January 2020 alone.[143] A further publication used a metapopulation model to generate an estimate with 95% CrI suggested that 75,815 individuals (95% CrI: 37,304–130,330) had been infected in Wuhan by 25 January 2020.[144]

These early phase estimates for SARS-CoV-2 infection are much larger than the known confirmed cases at the time.[145] It is unknown if we had detected the outbreak at a much smaller size if impact reduction or even containment may have been achievable, such as was the case for SARS-CoV-1.[146] If so, the COVID-19 pandemic has demonstrated the immense social, economic, and public health consequences of delayed pathogen detection. One major factor that differentiates SARS-CoV-1 and SARS-CoV-2 is that the latter has more potential for asymptomatic transmission, which is known to make an infectious disease outbreak less controllable.[147] However, early detection through population-level monitoring could potentially assist in identifying asymptomatic transmission chains and lead to containment if response actions begin while the outbreak is still small. This provides an imperative to explore the development of surveillance systems capable of detecting novel pathogens at an early stage of the outbreak to prevent a similar in the future.

3.2.2 Current Detection for Novel Pathogens

The average time-to-detection for novel pathogen outbreaks is not known with certainty. In research examining detection delays for the wider category of all emerging infectious diseases (EIDs), between 1996 - 2014 the global median time from outbreak start to detection was found to be 20 days (95% CI: 16–25 days) and to laboratory confirmation to be 36 days (95% CI: 32–46 days).[74] This study defined the outbreak's start as the first symptom onset or hospitalisation date, depending on the available data. This implies that the actual outbreak start date is likely even earlier if defined by the initial case exposure, representing the time point when the first infection begins. The delay experienced in detecting outbreaks caused by novel pathogen EIDs is potentially even longer and warrants investigation given the public health risks posed by this EID subset.

In Chapter Two, we explored the current surveillance infrastructure for novel diseases in 195 countries and determined that only a minority (73/195, 37.4%) have any public evidence of systems in place for detecting such outbreaks. For those that do, most countries rely on passive indicator-based surveillance (IBS), where public health authorities are alerted to symptomatic cases presenting to a health service.[148] Detection thresholds range from reporting the first case of a suspected new disease in a hospital up to five simultaneous cases, with some countries also having one or two deaths as a trigger for reporting suspected novel diseases to public health authorities. The different novel disease detection threshold criteria for reporting found in Chapter Two are provided in Table 3.1, including examples of countries where these thresholds apply.

Table 3.1. Different detection threshold criteria for novel disease reporting in national public health surveillance systems with an explanation of the requirement and example countries provided. In this context, a "case" refers to a symptomatic infected person presenting to a health service.

Threshold	Explanation	Example Countries	Reference
1 case	Reporting required for any disease of unknown aetiology	Argentina, Russia	[149,150]
2 cases	Cluster of 2 or more cases in an unknown disease outbreak	Liberia, Senegal	[151,152]
3 cases	3 or more cases or more of a disease of unknown aetiology	Oman	[153]
4 cases	Stage of SARS-CoV-2 outbreak discovery	China*	[96]
5 cases	Unknown disease with 5 or more patients hospitalised	Latvia	[154]
1 dead	Death due to an unknown transmissible pathogen	Chile, India	[155,156]
2 dead	Cluster of 2 or more deaths due to an unknown disease	Bhutan	[157]

* This threshold is included as a "real-world" scenario of the number of confirmed cases when a novel pathogen outbreak was detected. Four cases of pneumonia of unknown aetiology were reported at the beginning of the SARS-CoV-2 outbreak.[96] However, China has a requirement for notification of a single case of an unknown disease,[158] and also specifies reporting requirements for any one case of pneumonia of unknown aetiology.[159]

These thresholds do not necessarily mean that a new disease will be detected once the criteria are met; indeed, as discussed in Chapter One, there has often been a significant delay to novel disease detection in historical pathogen emergence scenarios.[84,107,108] Even in the idealised scenario where detection and reporting occur immediately upon the criteria being met, it is currently unknown what the impacts are of these different detection thresholds on time and the total case number at discovery. Given the heterogeneity of approaches, it is crucial to understand the potential delay and size of a novel disease outbreak by the point it is detected and known to public health authorities.

As discussed in Chapters One and Two, novel pathogen outbreaks can also be detected through other modalities. In particular, event-based surveillance (EBS) is a proactive approach to detecting emerging disease threats by monitoring unusual events, rumours, and other sources of information.[44] For example, the first signals of an outbreak could come from informal reports of cases or deaths, or be detectable through analysing internet search

terms.[45,46] EBS can complement passive IBS systems and has the potential to identify novel pathogen outbreaks before they are detected through conventional methods. However, only a minority of nations operate any form of EBS, and even fewer conduct data analysis daily.[48] In addition, most forms of EBS rely on symptomatic cases to generate the signal, inherently limiting when the outbreak can be detected if there is a sizeable proportion of asymptomatic infections.

To illustrate the effects of the current systems of detection for novel pathogens, Figure 3.1 presents a fictional scenario in which a novel pathogen with a basic reproductive number ($\mathcal{R}_0$) of 3 initiates from a point source outbreak with a single person initially exposed and infected. In this scenario, the novel pathogen could be natural, accidental or deliberate in origin. As illustrated, the majority of infected individuals develop mild symptoms, with one-third remaining asymptomatic but still capable of transmission. Severely symptomatic cases constitute one-in-nine infected persons, and it is possible that some of these may engage in health behaviours such as buying over-the-counter medication or using an online search engine to investigate their symptoms, which could potentially be detected using EBS. For simplicity, all infected individuals are presumed to be equally infectious regardless of the presence of symptoms or disease severity. In this fictional scenario, only one-third of severely symptomatic individuals present to health services, and it is from these cases that the outbreak can be detected through traditional IBS in countries with reporting requirements for novel diseases.

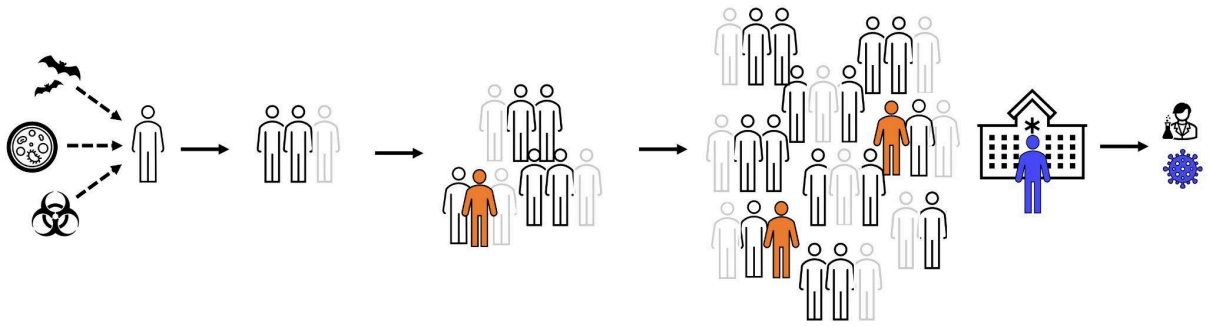


Figure 3.1 Fictional illustration of a point source outbreak of a novel pathogen with a $\mathcal{R}_0$ of 3 commencing from an unknown cause that could be natural, accidental or deliberate in origin. Black outlined figures represent infectious cases with mild symptoms, while grey figures represent asymptomatic infectious cases. Orange figures develop more severe symptoms, enough that they may engage in health behaviours that could trigger event-based surveillance in aggregation. Blue figures develop severe enough symptoms that they present to a health service, where they could undergo clinical evaluation and testing which leads to pathogen identification and notification to public health authorities.

As can be seen through this illustration, the actual size of a novel pathogen outbreak could be much larger (40 total cumulative infections) than the number of cases known to public health authorities (one case). In addition, there could have been multiple generations of the epidemic before the outbreak was detected. The exact time delay and size at detection will be determined by a variety of factors, including the extent of asymptomatic and pre-symptomatic transmission, probability and rate of healthcare presentation, and disease notification practices. Stochastic effects will also have a strong predominance in this early stage, including whether the initial infections were in a vulnerable population more likely to develop symptoms and seek medical treatment. Given the potential shortcomings of reliance on healthcare presentation for outbreak detection, overcoming the limitations of current detection mechanisms and reducing the delay to outbreak discovery necessitates exploring a new approach to novel disease surveillance.

3.2.3 Future Detection

Recent innovations in novel pathogen detection could allow for more timely discovery of new outbreaks.[63,64] In particular, metagenomic next-generation sequencing (mNGS) could

soon play a crucial role in the early detection of novel pathogens.[62,67] This approach involves sequencing the genetic material from environmental or clinical samples, which enables the identification of a wide range of pathogens, including viruses and bacteria. By analysing the entire genetic content of a sample without an *a priori* hypothesis of the causative agent, mNGS has the potential to detect both known and novel pathogens, providing a powerful tool for identifying emerging diseases in an outbreak. This has led to calls for incorporating mNGS into global early warning systems to prevent novel pathogen pandemics.[65,67,160,161]

In the context of surveillance for novel diseases, mNGS can be applied to various sample types, such as from wastewater, potential animal reservoirs and human clinical specimens. For instance, wastewater surveillance can provide an early warning system for community outbreaks, as the presence of a pathogen in wastewater often precedes the identification of clinical cases.[162] Even when a pathogen genetic sequence is new, detection of exponential growth of a previously unrecognised genome over subsequent sampling time points could be a reasonable assumption during an outbreak in the early stages at the time of discovery.[137] By integrating mNGS into a comprehensive surveillance strategy, public health authorities can enhance their capacity for early detection of novel pathogens, thus improving outbreak response and reducing the likelihood of larger-scale epidemics and pandemics.

3.2.4 Potential Surveillance Strategies

Recent advances in metagenomics have paved the way for pathogen-agnostic approaches to infectious disease surveillance. This unbiased detection method could allow for more comprehensive and active surveillance, significantly improving the chances of early identification and containment of novel pathogens. However, because mNGS technology is new, there has not been extensive research completed on sampling strategies for novel disease

detection in the wider population. Previous population-based surveillance studies for known diseases, highlighted below, can provide some insights into how this new approach could be incorporated and if the required testing level would be feasible.

Some outbreak detection approaches used for animal populations include random or targeted sampling in high-risk populations can offer insights.[163,164] Random sampling involves testing a subset of individuals from the population without bias, ensuring that each individual has an equal chance of being selected. This provides a representative snapshot of the population. In targeted sampling, specific subpopulations or locations are prioritised based on risk factors, which has the benefit of increasing the likelihood of detecting infections. However, it would be hard to predict the risk factors for a currently unknown disease, and targeted sampling, therefore, risks excluding infected members of the population. One study comparing early detection surveillance in animal populations found that population coverage, sampling frequency and test sensitivity were the most important factors for an early warning system.[165]

The potential benefits of population-based surveillance are highlighted in Figure 3.2. This illustration depicts the infection pyramid for a novel disease outbreak emergence. As shown, in many scenarios, infections in the population can be potentially missed at each step in the pyramid. Current surveillance for outbreak detection relies upon clinicians suspecting a novel disease, conducting tests and reporting to public health authorities. The detection threshold for notification varies between jurisdictions, as outlined in Table 3.1. With a well-functioning health system, sensitive tests and a strong public health workforce, ideally few infections will be missed after clinical suspicion takes place. However, with current surveillance, it is possible that several infections remain undiscovered before healthcare presentations occur. Active surveillance through monitoring could potentially detect infections in the population

before cases are discovered by hospital admissions and clinical suspicion. It could also potentially detect diseases before an EBS signal is recognised, as most EBS symptoms rely upon symptomatic cases as well, although through a different detection mechanism.

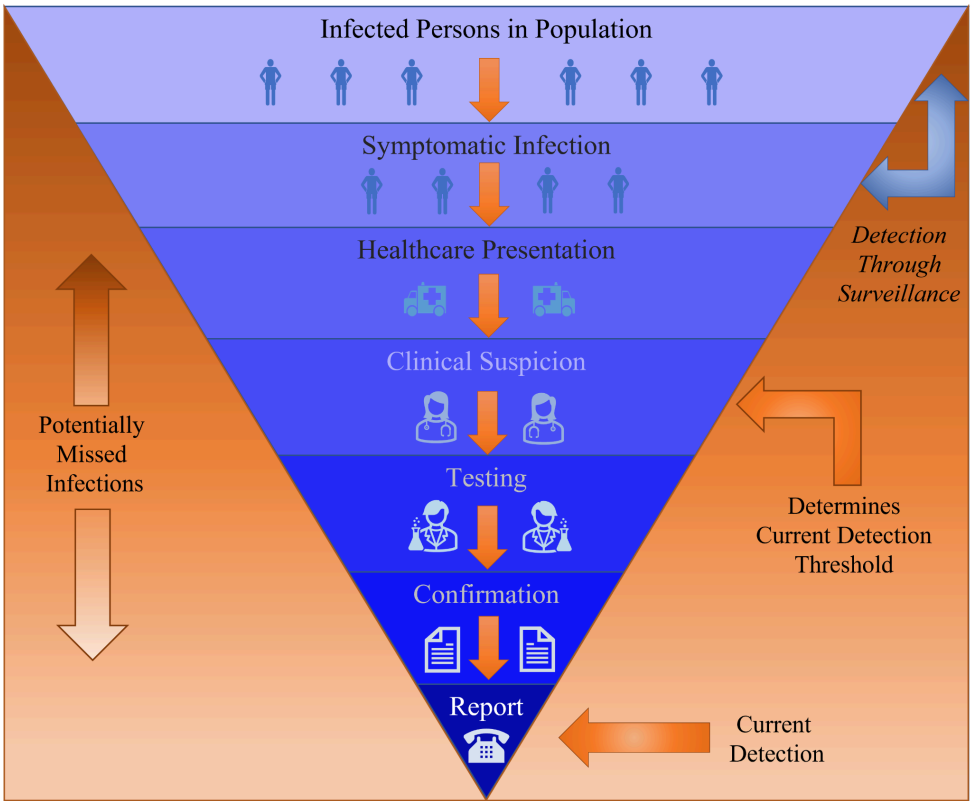


Figure 3.2 Diagram of an inverted infection pyramid, with the total number of infected persons for a novel disease at the top and each step before the disease is reported to public health authorities at the bottom. At each step, there is the potential to miss infections, represented in orange (darker orange background depicts more potentially missed infections, with lighter orange depicting fewer). Current detection happens at the bottom, after a detection threshold determined by the number of cases or deaths in a particular country necessary to trigger novel disease clinical suspicion and reporting. Detection through surveillance, represented with the blue arrow and italics, could potentially detect the outbreak amongst infections that may have been otherwise missed.

Implementing pathogen-agnostic surveillance programs that incorporate random sampling within the population could potentially facilitate faster detection of emerging diseases. By removing the need for prior knowledge of a specific pathogen, this approach could reduce the time lag between the emergence of a novel disease and its identification, enabling more timely public health interventions. However, it is currently unknown what level of surveillance coverage, or number of tests in the population, would be required to detect a

novel disease prior to current surveillance, and therefore if it is feasible. It is also unknown how testing coverage levels need to change with different disease transmission dynamics.

3.2.5 Research Questions

In this Chapter, we present a stochastic model based on the transmission dynamics of SARS-CoV-2 to investigate the early phase of a novel pathogen outbreak. By exploring the different outbreak detection thresholds used by countries, we aim to understand the average time-to-detection, the size of the outbreak at discovery, and the potential for active population surveillance through random sampling to result in earlier detection. We use a sensitivity analysis to determine how applicable the results could be to diseases other than SARS-CoV-2 with different transmission dynamics. Overall, this Chapter aims to address the following research questions:

1. For different novel disease outbreak detection thresholds used by countries, what is the time-to-detection for a SARS-CoV-2 outbreak?
2. What is the expected total epidemic size at the time of detection?
3. What level of surveillance coverage would be required to detect the outbreak before the first case presents to a hospital, or within seven, 14 or 28 days?
4. How do our results differ for diseases with different transmission dynamics?

Section 3.3 Methodology

3.3.1 Model Overview

In this study, we employed a stochastic SEPIR-AHD model to simulate the transmission dynamics of a novel pathogen in a population. The SEPIR-AHD model is a compartmental model that classifies the entire population into eight classes: susceptible (S), exposed (E), pre-symptomatic infectious (P), infectious symptomatic (I), removed (R), asymptomatic infectious (A), hospitalised infectious (H), and dead (D). Each of these compartments was chosen for inclusion because of heterogeneity in the disease stages in which the outbreak can be detected in current or future surveillance. Four of these compartments (P, I, A and H) contain individuals who are infectious. The R class represents when an individual ceases to be infectious; this can be synonymous with recovery, as defined by the cessation of symptoms. However, because SARS-CoV-2 can result in asymptomatic infections, or have persistence of symptoms beyond the period of transmissibility, this model considers the R compartment from the perspective of cessation of infectivity.[166,167]

The schematic for the model is shown below in Figure 3.3, with the events at each step and their effects shown in Table 3.2. Current early detection surveillance, as outlined in Table 3.1, can potentially identify infections from the H and D compartments. Future surveillance strategies could potentially detect infectious individuals in the P, A and I compartments. Exposed individuals, while infected, are typically not able to be identified as cases through either mechanism because they are not showing symptoms, nor do they have a high enough pathogen load for tests to return a positive result. Therefore, those in the exposed class are assumed to have undetectable infections.

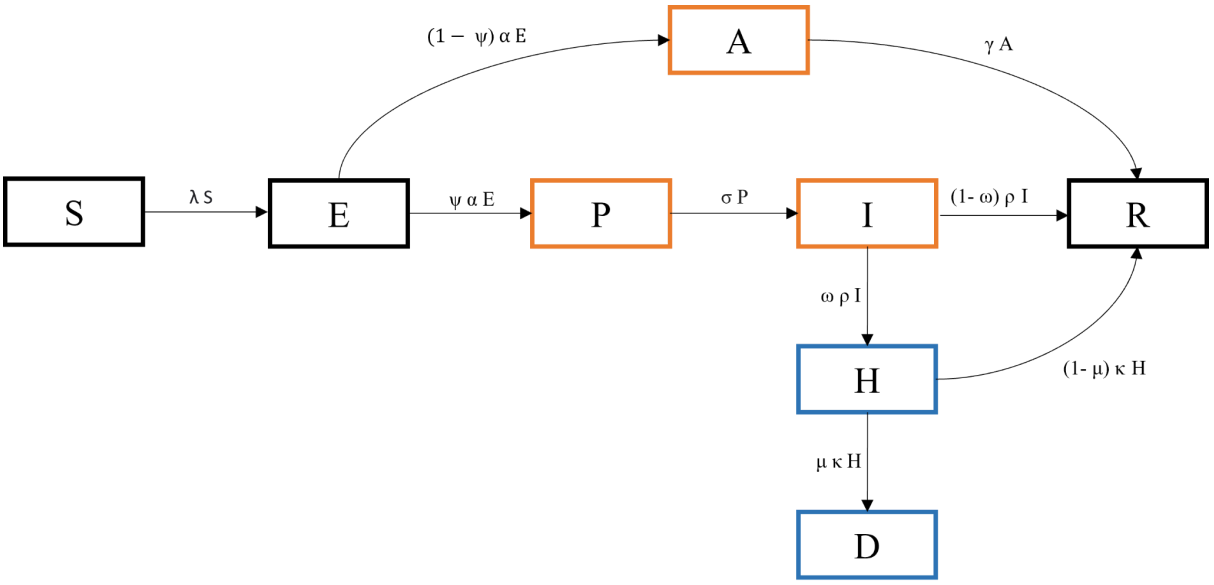


Figure 3.3 Schematic of the compartmental model with rates and flow direction depicted. S: Susceptible; E: Exposed; P: Pre-symptomatic infectious; I: Infectious symptomatic; R: Removed; A: Asymptomatic infectious; H: Hospitalised infectious; D: Dead. In blue are the compartments where current surveillance can potentially detect infections at the beginning of a novel pathogen outbreak. In orange are the additional compartments where future population-based surveillance may be used to detect infections.

Table 3.2 Outline of all the events in the stochastic SEPIR-AHD model, their effect, rate and probability of an event in each time step. S: Susceptible; E: Exposed; P: Pre-symptomatic infectious; I: Infectious symptomatic; R: Removed; A: Asymptomatic infectious; H: Hospitalised infectious; D: Dead.

Event	Effect	Rate	Probability in [t, t + δt]
Transmission	S → S - 1, E → E + 1	λS	$\lambda S \delta_t$
Presymptomatic Infectiousness Onset	E → E - 1, P → P + 1	$\psi \alpha E$	$\psi \alpha E \delta_t$
Symptomatic Infectiousness Onset	P → P - 1, I → I + 1	σP	$\sigma P \delta_t$
Asymptomatic Infectiousness Onset	E → E - 1, A → A + 1	$(1 - \psi) \alpha E$	$(1 - \psi) \alpha E \delta_t$
Hospitalisation	I → I - 1, H → H + 1	$\omega \rho I$	$\omega \rho I \delta_t$
Death	H → H - 1, D → D + 1	$\mu \kappa H$	$\mu \kappa H \delta_t$
Symptomatic Removed	I → I - 1, R → R + 1	$(1 - \omega) \rho I$	$(1 - \omega) \rho I \delta_t$
Asymptomatic Removed	A → A - 1, R → R + 1	γA	$\gamma A \delta_t$
Hospitalised Removed	H → H - 1, R → R + 1	$(1 - \mu) \kappa H$	$(1 - \mu) \kappa H \delta_t$

The force of infection (λ) for the model, representing the rate at which susceptibles contract the disease, is given in Equation 3.1. Frequency-dependent transmission, where the contact rate between infectious and susceptible persons does not depend on population density, was assumed in the model.

$$\lambda = \frac{\beta_P P + \beta_I I + \beta_A A + \beta_H H}{N} \quad (3.1)$$

The model was implemented using continuous-time Markov chains (CTMC) through the Gillespie algorithm, which is a Monte Carlo simulation method.[168] The Gillespie algorithm ensures that the model accurately represents the random nature of individual-level processes by simulating which discrete event (Table 3.2) occurs based on probabilities proportional to its rate. CTMCs allow transition events to occur at any stage through continuous time steps, with the rate exponentially distributed. To simulate the stochastic SEPIR-AHD model using the Gillespie algorithm, we utilised the SimInf package.[169] We conducted 100,000 simulations in a population of 100,000 individuals over 600 days, with each simulation starting with one exposed person. We repeated the results with different population sizes, from 10,000 persons up to 8,266,000 persons (the 2019 population of Wuhan, China).[170] However, because of the use of frequency-dependent transmission, our results were widely robust to changes in population size, and thus results for the original population choice were presented.

In addition to the stochastic SEPIR-AHD model, we also deployed a deterministic model with the same compartmental structure for comparison. The deterministic model used ordinary differential equations (ODEs) to describe the transitions between compartments, allowing us to analyse the behaviour of the system without considering the random fluctuations inherent in individual-level processes. The ODEs for the deterministic model are depicted as a set below in Equations 3.2.

$$\frac{dS}{dt} = -\lambda S$$

$$\frac{dE}{dt} = \lambda S - \alpha E$$

$$\frac{dP}{dt} = \psi \alpha E - \sigma P$$

$$\frac{dI}{dt} = \sigma P - \rho I$$

$$\frac{dR}{dt} = \gamma A + (1 - \omega) \rho I + (1 - \mu) \kappa H$$

$$\frac{dA}{dt} = (1 - \psi) \alpha E - \gamma A$$

$$\frac{dH}{dt} = \omega \rho I - \kappa H$$

$$\frac{dD}{dt} = \mu \kappa H \quad (3.2)$$

3.3.2 Next-Generation Matrix and Parameters

To estimate the transmission coefficient or effective contact rate (β) for our models, we employed the next-generation matrix method. First, the expression for $\mathcal{R}_0$ was derived in Matlab with the Symbolic Math Toolbox using the method initially described by Diekmann et al and later summarised.[32,171] This method deploys the following steps:

1. Find all *n* infected model compartments (In our model there are five: Exposed, Pre-symptomatic, Infectious symptomatic, Asymptomatic, Hospitalised)
2. For all entirely new infections entering each *n* compartment, construct a *n* x 1 matrix (**F**), excluding infection transfers from other compartments
3. For all infection transfers to each *n* compartment, construct a *n* x 1 matrix ($\mathbf{V}^+$)
4. For all infection losses leaving each *n* compartment, construct a *n* x 1 matrix ($\mathbf{V}^-$)
5. Construct a *n* x 1 matrix (**V**) where $\mathbf{V} = \mathbf{V}^- - \mathbf{V}^+$
6. Generate two *n* x *n* Jacobian matrices **f** and **v** that are with respect to the *n* infectious state variables the partial derivatives of **F** and **V**
7. Evaluate the Jacobian matrices at the disease-free equilibrium (dfe), with $\mathcal{R}_0$ being the greatest eigenvalue of $\mathbf{fv}^{-1}|_{\mathbf{dfe}}$

Using this method, the derived expression for $\mathcal{R}_0$ is as below in Equation 3.3.

$$\mathcal{R}_0 = \frac{\beta_P \psi}{\sigma} + \frac{\beta_I \psi}{\rho} - \frac{\beta_A (\psi - 1)}{\gamma} + \frac{\beta_H \omega \psi}{\kappa} \quad (3.3)$$

Many factors can affect the pre-symptomatic transmission rate (β_P), the symptomatic infectious transmission rate (β_I), the asymptomatic transmission rate (β_A) and the hospitalised transmission rate (β_H) in reality. For example, hospitalised cases may have higher pathogen loads making them more contagious, but be less ambulatory and thus in contact with fewer susceptible persons. Asymptomatic cases may have the most number of contacts, but each contact may be less likely to transmit due to a lower pathogen load. Presymptomatic cases sometimes can be more or less infectious than symptomatic cases depending on a variety of disease factors. For simplicity, it was decided to include the overall effective contact rate (β) as equivalent for cases in the four infectious classes, as below.

$$\beta = \beta_I = \beta_P = \beta_A = \beta_H$$

For SARS-CoV-2, the mean $\mathcal{R}_0$ was estimated to be 3.28 in 2020.[103] After rearranging Equation 3.3, the equation was solved for β using the *uniroot* function. This gave a β of 0.24.

The length of the infectious period in SARS-CoV-2 is known to depend on symptom development and severity, requiring account for in model parameterisation.[172] Because our model is based on transmission dynamics, relevant factors included whether an individual is infectious and the duration of infectivity in each of the compartments. The average duration of time an individual spends in the symptomatic infectious (I) and hospitalised (H) classes can be determined using the law of total expectation. As shown in Equation 3.4, the average expected time an individual spends in the symptomatic infectious (I) class (ρ^{-1}) depends on the mean time to hospitalisation once symptomatic infectious (χ), the mean time to recovery

in the non-hospitalised symptomatic infectious (ξ), and the probability of hospitalisation given symptoms (ω).

$$\rho^{-1} = \chi\,\omega + \xi\,(1 - \omega) \tag{3.4}$$

For SARS-CoV-2, χ and ξ have been estimated in the literature to be 5.74 days and 13.4 days, respectively.[172,173] The overall hospitalisation probability has been reported as 1.92%, including all symptomatic and asymptomatic SARS-CoV-2 infections.[174] Asymptomatic cases, when excluding pre-symptomatic individuals who later proceed to have symptoms, have been estimated in a systematic review and meta-analysis to represent 35.1% of all SARS-CoV-2 infections.[166] This implies that the overall probability of developing symptoms (ψ) is 64.9%. Given only symptomatic cases are hospitalised for their infection, this implies the probability of being hospitalised given symptoms (ω) to be 2.96%. Using these values in Equation 3.4 gives a ρ^{-1} of 13.2 days. The inverse of ρ^{-1} is the rate at which individuals, on average, leave the symptomatic infectious class, with ρ = 0.076.

Similarly, the expected average time an individual spends in the hospitalised (H) class (κ^{-1}) can be calculated using Equation 3.5, using the average time period of infectivity in hospital in those that survive (θ), the average time period of infectivity in hospital non-survivors (η), and the probability of death given hospitalisation (μ).

$$\kappa^{-1} = \eta\,\mu + \theta\,(1 - \mu) \tag{3.5}$$

For SARS-CoV-2, the average length of infectiousness, as determined through serial analysis of detected viral shedding, has been reported in both surviving and non-surviving hospital patients from the point of their symptom onset, but not from the date of hospitalisation.[175] For the purposes of our model, θ can be inferred by subtracting χ from the average length of

infectiousness from symptom onset to cessation of infectiousness in hospital survivors (20.0 days), giving 14.3 days.[175] Similarly, η can be derived by subtracting χ from the reported average length of infectiousness from symptom onset to cessation in hospital non-survivors (18.5 days), giving 12.8 days.[175] This value estimate for η is close to a February 2020 study reporting the time between hospital admission and death to be 13.0 days (95% CI: 8.7 - 20.9), suggesting infectivity can be assumed to persist until the time of death in SARS-CoV-2 infection non-survivors.[176] The value for μ in a systematic review and meta-analysis has been estimated as 0.17.[177] Using these values in Equation 3.5 gives a κ^{-1} value of 14.0 days. The inverse of κ^{-1} represents the average rate at which individuals leave the hospitalised class, with $\kappa = 0.071$. Table 3.3 describes all the SARS-CoV-2 parameters used in the model. Parameters were chosen for early COVID-19 transmission dynamics to model wild-type SARS-CoV-2 using estimates from the literature.

Table 3.3 Disease parameters for the SARS-CoV-2 model with the definition, value and references for each.

Parameter	Definition	Value	Reference
β	Transmission coefficient; effective contact rate	0.24	Derived*
α	Rate of progression to infectiousness; (mean latent period)$^{-1}$	(3.3 days)$^{-1}$ = 0.30	[178]
ψ	Probability of developing symptoms	0.65	[166]
1 - ψ	Probability of remaining asymptomatic	0.35	[166]
σ	Symptom development; (mean incubation - latent period)$^{-1}$	(2.6 days)$^{-1}$ = 0.38	[179]
ω	Probability of hospitalisation given symptoms	0.019	[174]
1 - ω	Probability of no hospitalisation given symptoms	0.98	[174]
ρ	Mean rate of leaving symptomatic infectious class	(13.2 days)$^{-1}$ = 0.076	Derived†
μ	Probability of non-survival given hospitalisation	0.17	[177]
1 - μ	Probability of survival given hospitalisation	0.83	[177]
κ	Mean rate of leaving hospitalised class	(14.0 days)$^{-1}$ = 0.071	Derived‡
γ	Mean asymptomatic infectious period^{-1}	(9.5 days)$^{-1}$ = 0.11	[180]

* Derived using the next-generation matrix method with an $\mathcal{R}_0$ of 3.28 using Equation 3.3
† Derived using Equation 3.4
‡ Derived using Equation 3.5

3.3.3 Assumptions and Definitions

In all modelling studies, certain assumptions need to be made regarding the disease dynamics and model structure. In this Chapter, we assumed all outbreaks commenced with a single exposed individual and that transmission in the population was frequency-dependent. Given the relatively fast transmission dynamics of SARS-CoV-2 infections, demographic effects such as births, immigration and natural deaths were excluded from the model. For detection thresholds involving hospitalised cases and deaths, we assumed detection occurred on the day of the criteria being met. For example, this could include when the first case entered the hospitalised compartment. This is idealised, as real-world detections of the outbreak will likely include delays due to diagnostic testing and reporting to public health authorities.[74] To address this, our detection threshold included a "real-world" scenario relevant to the COVID-19 pandemic, where detection takes place at ≥ four cases in hospital as listed in Table 3.1. Surveillance for infectious diseases can also occur in non-hospital healthcare settings and EBS, but because of the variability in testing availability and reporting rates, our model only considered hospitalised cases and IBS. Deaths could also potentially occur in the community in various outbreak scenarios, but for simplicity this mechanism to outbreak detection was excluded from our model.

Outbreak extinction was defined as occurring when there were no persons remaining in the infected compartments, with the full population in the susceptible, removed or dead classes. "Small outbreaks" were defined as all outbreaks that went extinct prior to any hospitalised case. Outbreaks with one or more hospitalised cases were designated as "large outbreaks" for the analysis. "Total outbreak size" was defined as the cumulative number of cases in the exposed, pre-symptomatic, infectious symptomatic, hospitalised, dead, recovered and

asymptomatic compartments. This was equivalent at any point in time to the population size minus the number of susceptibles.

3.3.4 External Comparison

To externally evaluate the performance of our SEPIR-AHD model, we compared its outputs with the estimates from a January 2020 study conducted by Imperial College London.[142] The authors estimated that there had been 1,723 symptomatic cases (95% CI: 427-4,471) of SARS-CoV-2 infections in Wuhan with symptom onset by 12 January 2020. This estimation was derived from a different approach than used in our study, which considered the international travel volume from Wuhan and the number of confirmed cases outside of China. The Imperial study explicitly excluded asymptomatic cases from their analysis, so we excluded the counts of these types of infections from this portion of the results.

At the time of the Imperial study, there were 41 confirmed hospitalised cases, two deaths, and three international cases. Thirteen days earlier, on 30 December 2019, there had been four hospitalised cases in Wuhan and zero deaths.[96] To compare the performance of our model with the Imperial estimate, we considered three metrics: the day of the outbreak, the number of symptomatic cases, and the number of reported deaths. We utilised the 2019 population of Wuhan, China of 8,266,000 persons for this portion of the study.[170] To determine the total number of symptomatic cases, we first divided the removed (R) compartment into three portions: R_H for hospitalised removed, R_A for asymptomatic removed, and R_I for infectious symptomatic removed, so each could be separately tracked. “Symptomatic cases” were defined as the total number of infected individuals who had shown symptoms, including those in the infectious symptomatic (I), hospitalised (H), dead (D), hospitalised removed (R_H) and infectious symptomatic removed (R_I) compartments by a given day. After running

100,000 simulations, we reported the median and interquartile ranges (IQRs) for outbreak day, number of hospitalised cases, number of deaths, total outbreak size and number of symptomatic cases.

3.3.5 Sensitivity Analysis

Our model, as constructed, returns results specific to SARS-CoV-2 transmission dynamics. To account for the variability in different disease parameters and the potential impact of these variations on our model outcomes, we performed a two-way sensitivity analysis. This involved generating different values for model parameters and comparing across a range of $\mathcal{R}_0$ values. For all rates included in the model, 1,000 simulations were run from 1 - 40 days with a time step of 1 day. For all probabilities, comparisons were made between 2.5 - 100% with a step of 2.5% for each parameter. These were compared against $\mathcal{R}_0$ values from 1.1 - 10 with a step of 0.1 for each, with Equation 3.3 solved for β for each $\mathcal{R}_0$ value. The $\mathcal{R}_0 \leq 1$ were not included because of the altered disease dynamics at these values and the aim of assessing results in propagating epidemics.[181] By simulating outbreaks with different parameter combinations, we were able to evaluate the days-to-detection and the size of the outbreak at the time of discovery for a variety of disease scenarios, including those with characteristics different from SARS-CoV-2. This analysis allowed us to assess the robustness of our model and better understand the factors that influence the early detection of novel pathogen outbreaks.

3.3.6 Public Health Interventions

To assess the impact of public health interventions, we adapted the deterministic SEPIR-AHD model to simulate different scenarios that involved isolating cases. The deterministic model was considered appropriate for this stage of the outbreak, as the number

of infected individuals was large enough for a deterministic approximation. We first initialised the deterministic model using the median values for each compartment obtained from the stochastic simulation outputs on the day that 50% of all large outbreaks would have been detected using ≥ one case in the hospital as the detection threshold.

We simulated three public health intervention scenarios: no intervention, isolate all hospitalised cases, and isolate all hospitalised and symptomatic cases. The latter represents the scenario in which public health advice is given to the population for all persons with symptoms to isolate at home. For simplicity, isolation was assumed to be 100% effective and reduced the compartment's respective value of β to zero. For each scenario, we calculated the final epidemic size, peak epidemic size, time to peak, peak healthcare caseload, total deaths, and the reproductive number. We also determined the extent to which effective contacts in the presymptomatic and asymptomatic populations needed to be reduced in order for the effective reproductive number ($\mathcal{R}_e$) to fall below one. This is because when the $\mathcal{R}_e < 1$, the disease incidence will decrease and the outbreak will continue to extinction over time.[182]

3.3.7 Surveillance Strategies

To assess the effectiveness of different surveillance coverage strategies, we used the hypergeometric distribution to determine the likelihood of outbreak detection on a given day for each simulation. The sampling frequency of one day was chosen because of previous research highlighting that short regular intervals between sampling are more important for outbreak detection than larger sample sizes for fast-moving epidemics.[137] For our study, we used the hypergeometric distribution to determine the number of people that need to be sampled each day at random from the population to detect the outbreak on or before the first hospital presentation and day seven and fourteen of the outbreak.

In contrast to the binomial distribution, the hypergeometric distribution models the discrete probability of an event from a finite population without replacement, meaning that each randomly sampled person is not able to be tested more than once per day. For large population sizes (N) with small levels of surveillance testing (s), such that $s \ll N$, the hypergeometric distribution approximates the binomial.[183] However, because we aimed to test a range of testing coverage where this assumption may not hold, the hypergeometric distribution was deemed appropriate for our research.

To investigate surveillance coverage levels, we used the *dhyper* function to compute the discrete probability mass function of the hypergeometric distribution, allowing us to estimate the probability of the outbreak remaining undetected ($P_{no\ case\ detected}$) through not a single case detected. We then used the complement rule ($1 - P_{no\ case\ detected}$) to determine the probability of detection of at least one case, given the number of tests used per day, the population size, and the number of days that had elapsed. The number of tests divided by the population size gave us the surveillance coverage levels needed to detect the outbreak on that day or sooner at a certain level of probability.

For computational efficiency, we employed a binary search algorithm instead of a serial search to determine the number of tests needed. The binary search algorithm is an efficient search method that can operate on a sorted array of elements. Also referred to as the divide-and-conquer algorithm, this strategy iteratively divides the search interval in half, comparing the target value with the middle element at each step.[184] For our research, the target value was the first (minimum) day at which 50% of the outbreaks will have been detected for a given surveillance coverage level. When the target value matches the middle

element, the search is successful. If the target value is less or greater than the middle element, the search interval is updated to the lower or upper half, respectively, discarding the irrelevant portion. This process continues until the target value is found. The binary search algorithm is highly efficient, as it reduces the search interval by half with each iteration, resulting in a logarithmic time complexity of $O(log\ n)$, where n is the number of elements in the array. This makes binary search significantly faster than linear search, which has a linear time complexity of $O(n)$. Using this approach, we could efficiently estimate the coverage level needed in a 100,000 person population at the granular level of one test per person per day.

The day of detection and size at outbreak discovery were reported for different coverage levels, ranging from 0.001% of the population (representing one person tested per day at random in a population of 100,000) up to 100% coverage. Small outbreaks, which went extinct prior to any hospital presentation, were excluded from this portion of the analysis. For simplicity, testing was assumed to have 100% sensitivity and specificity and take place at random in the population without replacement, ignoring the complexities introduced by false positive and false negative results. The minimum surveillance coverage level needed to detect 25, 50, 75 and 90% of outbreaks before the first case presentation to hospital were determined across a range of disease parameters. This represents what would be required to surpass the most optimal form of current surveillance detection. Because some public health systems may desire a time target instead of beating the current system of detection, these results were repeated with a 7, 14 and 28-day goal of detecting the outbreak since the first case exposure event.

As this study did not involve human research subjects, ethics approval was not required.

Section 3.4 Results

3.4.1 Model Output

We ran 100,000 simulations of the stochastic model in a population of 100,000 persons over a time period of 600 days in RStudio with R Version 4.2.2.[122] For each compartment, the median trajectory outputs and IQRs for the first 300 days are shown in Figure 3.4. Out of 100,000 simulations commencing with a single exposed infected person, 26.19% of trajectories end without a single hospital presentation. We refer to these simulations as "small outbreaks" as they have a median size of one infected person (IQR: 1 - 1) and a maximum of 17 total infections. In small outbreaks, epidemic extinction occurs at a median time of 8 days (IQR: 5 - 13 days). In Figure 3.4, we include the deterministic model output for comparison using the same parameter values and initial starting conditions. As can be seen, the deterministic model follows a similar trajectory to the median stochastic output, but with the growth phase shifted left to earlier time points before converging at later time points. This is due to the predominance of the inherent randomness within the stochastic model at the early stages leading to output differences, in particular, where some stochastic outbreaks become extinct in this early stage. The central limit theorem takes effect after the outbreak reaches a greater size given the large number of simulations. When small outbreaks are excluded, the median values of the stochastic model align with the outputs of the deterministic model.

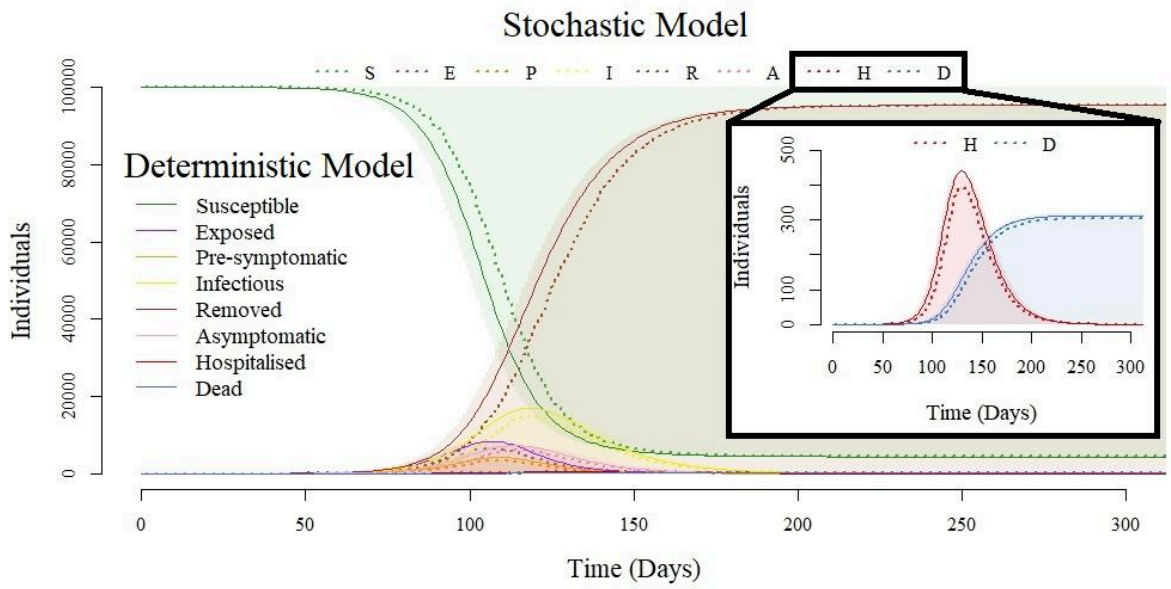


Figure 3.4 Stochastic model output of the number of individuals in each compartment over the first 300 days after running 100,000 simulations, with median (dotted line) and IQR (shaded area) for each compartment after seeding with a single exposed person in a population of size 100,000. The deterministic model outputs (solid lines) are also depicted for comparison with the same parameter values and initial starting conditions. On the right, displayed is a zoomed plot of the Hospitalised (H) and Dead (D) compartments, which are the two compartments where infected cases can be detected in the early stages with current surveillance. S: Susceptible in green; E: Exposed in purple; P: Pre-symptomatic in orange; I: Infectious symptomatic in yellow; R: Removed in brown; A: Asymptomatic in pink; H: Hospitalised in red; D: Dead in blue; IQR: Interquartile Range.

For "large outbreaks" that persist until at least one hospitalisation, the median final epidemic size is 95,662 total infected individuals (IQR: 95,601 - 95,722), with a maximum of 96,015 in the 100,000-person population. Large outbreaks have a median epidemic duration of 301 days (IQR: 287 - 317) and a maximum of 477 days. The median peak size of large outbreaks is 35,191 infected persons (IQR: 35,029 - 35,350), occurring on day 113 (IQR: 107 - 121) since the initial infection. In 99.81% of the large outbreak simulations resulting in at least one hospitalisation, the epidemic continues on to at least two hospitalised cases. From these, 100% of the simulations continue to grow and meet the other detection thresholds outlined in Table 3.1, resulting in five or more hospitalisations and at least two deaths. This implies that once the outbreak reaches a single hospitalisation, in almost all simulations the epidemic continues to increase in size and eventually reach and surpass the other detection thresholds.

3.4.2 Outbreak Detection

We calculated the time between outbreak commencement and discovery for each of the detection thresholds specified in Table 3.1. Figure 3.5 depicts the cumulative probability of outbreak detection across all 100,000 simulations. As can be seen, the detection thresholds used by various countries result in different delays to discovery. However, these asymptote near the same outbreak detection probability, reflecting that 26.19% of outbreaks go extinct before at least one hospitalisation takes place, as was highlighted previously. This implies that around a quarter of small outbreaks may emerge and go extinct before detection occurs with current surveillance relying on hospital-based detection.

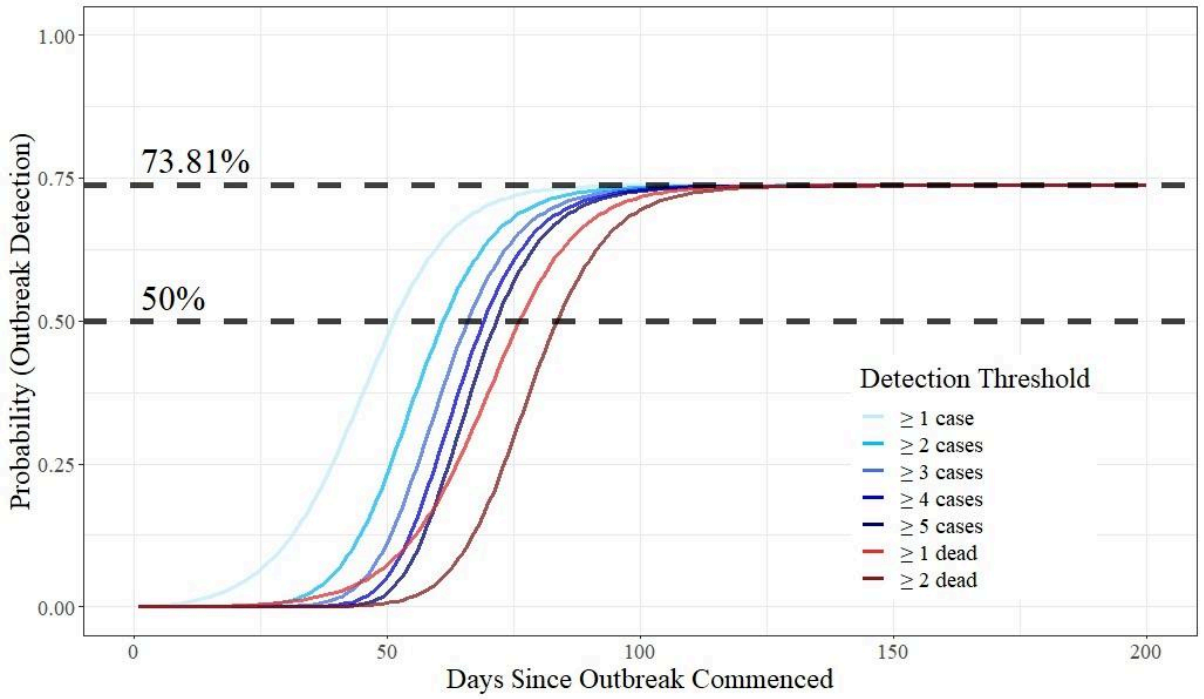


Figure 3.5. Cumulative probability of outbreak detection over time since the outbreak commenced. The seven different detection thresholds are depicted with colours as shown. Cases are the number of individuals in the hospitalised (H) class, while the dead are the number of individuals in the dead (D) class. The black dotted lines represent when 50% of outbreaks will be detected and the maximum probability (73.81%) of detection across all 100,000 simulations.

Figure 3.6 displays the cumulative probability of detecting the epidemic for all large outbreaks across the seven different detection thresholds. This plot is similar to Figure 3.5,

but after excluding small outbreaks that go extinct before at least one hospitalisation, there is a slight shift to the left to early discovery time points. As the number of cases or deaths required to trigger detection increases, so does the delay between outbreak commencement and detection. Interestingly, when the detection threshold is ≥ one death, the cumulative probability of detecting the outbreak on a given day initially exceeds that of some case-based detection thresholds, with the exception of ≥ one case in the hospital. This is because in a proportion of outbreak scenarios, a death can occur in one of the early hospitalised cases before, for example, four or five presentations.

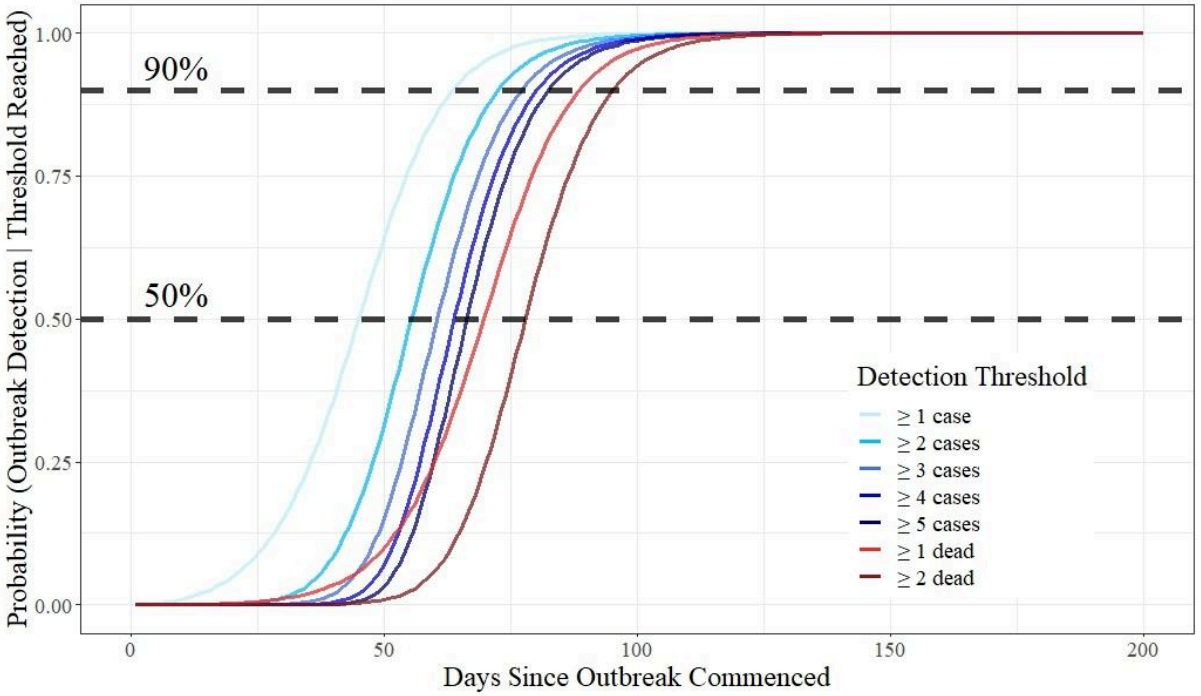


Figure 3.6. Cumulative probability of epidemic discovery over time comparing seven detection thresholds. Plotted are all large outbreaks where the threshold of at least one hospitalisation is met, which total 73,808 simulations. Cases are the number of individuals in the hospitalised (H) class, while the dead are the number of individuals in the dead (D) class. The black dotted lines depict when 50% and 90% of outbreaks will have been detected for a given detection threshold.

The cumulative probability of outbreak discovery for a given epidemic size is shown in Figure 3.7 for each of the seven detection thresholds. On the left, the total outbreak size is unscaled, while on the right, the x-axis has been $\log_{10}$ transformed. As can be seen, after

transformation, the size at detection follows a similar trajectory to the time-to-detection as seen in Figure 3.6. This is a reflection of the exponential growth in epidemic size over time. There is stochastic noise visible at lower total outbreak sizes. Because this plot considers total outbreak size, it was not necessary to exclude small outbreaks from the analysis.

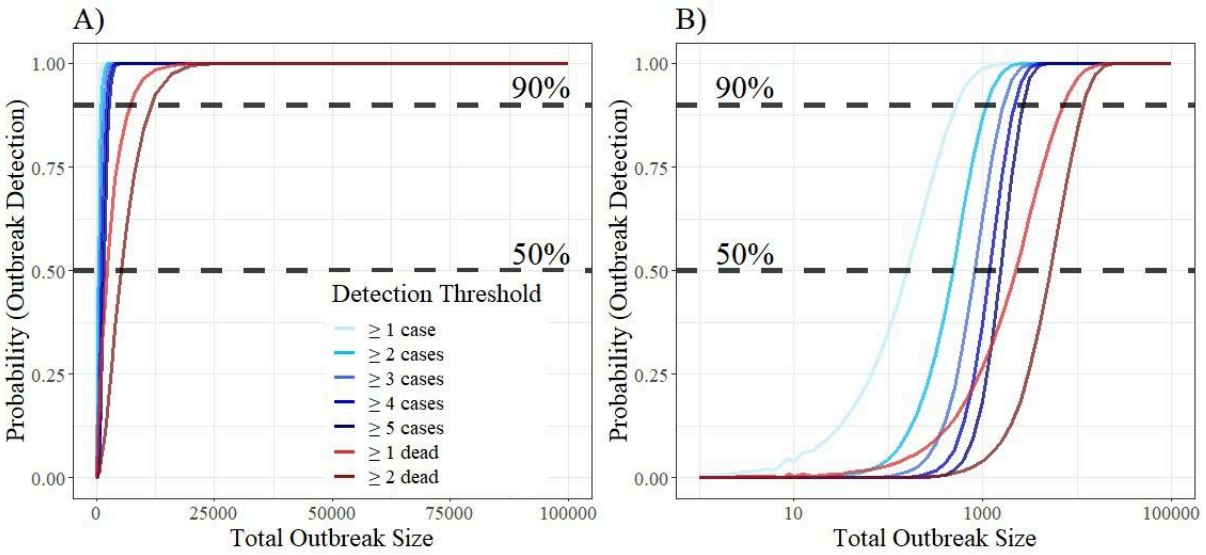


Figure 3.7. Cumulative probability of epidemic discovery over total outbreak size comparing seven detection thresholds for all 100,000 simulations. Cases are the number of individuals in the hospitalised (H) class, while the dead are the number of individuals in the dead (D) class. A) Unscaled original plot. B) Log_{10} transformed x-axis (total outbreak size). The black dotted lines depict at what outbreak size 50% and 90% of epidemics will have been discovered for a given detection threshold.

The summaries of time to outbreak detection and size at discovery for the different thresholds are shown in Table 3.4. As can be seen, 50% of outbreaks which result in one or more hospitalised cases will be detected by day 46. For the same detection threshold, 90% of outbreaks are detected by day 64. For each additional case added to the detection threshold, the time-to-detection increases, however, this becomes narrower with each step. For the threshold of ≥ one dead, 50% of outbreaks will be detected after ≥ five cases have presented to a hospital (70 vs 67 days) but the wider IQR for ≥ one dead indicates that 25% of outbreaks will be detected on day 60 for both when the threshold is ≥ one dead or ≥ five cases in hospital. As seen in Figure 3.6, at outbreak detection probabilities lower than 25%, the ≥

one dead detection threshold is more likely to result in discovery on a given day than ≥ five cases in hospital. This reflects the dynamics of the model, where in some trajectories an early hospitalised case dies before several presentations to a hospital have had a chance to occur.

Table 3.4. Time to outbreak detection since and size at discovery, for seven different detection thresholds. Cases are the number of individuals in the hospitalised (H) class, while the dead are the number of individuals in the dead (D) class. The median (IQR) represents when 50% (25-75%) of outbreaks have been discovered, with the values for when 90% of outbreaks have been detected also provided. IQR: Interquartile Range.

Detection Threshold	Time to Outbreak Detection (Days)		Outbreak Size at Detection (Total Infected)	
	Median (IQR)	90%	Median (IQR)	90%
≥1 case	46 (36 – 55)	64	155 (57 – 321)	540
≥2 cases	56 (48 – 64)	73	509 (294 – 806)	1,150
≥3 cases	61 (54 – 69)	78	879 (571 – 1,276)	1,716
≥4 cases	64 (58 – 72)	81	1,253 (874 – 1,734)	2,236
≥5 cases	67 (60 – 75)	83	1,635 (1,187 – 2,179)	2,757
≥1 dead	70 (60 – 80)	89	1,844 (474 – 4,103)	7,032
≥2 dead	78 (71 – 87)	96	5,509 (3,235 – 8,661)	12,173

The size at outbreak detection increases significantly across the different detection thresholds. As can be seen in Table 3.4, 50% of outbreaks will be detected at ≥ one case in a hospital when the epidemic size is 155 total infected. For the same threshold, 90% of outbreaks will have been detected by the point that 540 total have been infected. If the detection threshold is ≥ one dead, 50% of outbreaks will be detected when the outbreak size is 1,844 individuals, with 90% detected when the outbreak has reached a cumulative 7,032 individuals infected.

3.4.3 External Comparison

To compare our model with the Imperial College London 12 January 2020 estimate of 1,723 cumulative symptomatic cases (95% CI: 427-4,471), three metrics were used: the day of the outbreak, the total number of symptomatic cases since outbreak commencement, and the

number of reported deaths. In our model, 50% of outbreaks were detected with ≥ four hospitalised cases by day 64 of the outbreak as seen in Table 3.4. This implies a median outbreak commencement date of the index case on 27 October 2019 (IQR: 23 October - 6 November 2019). This aligns with two modelling studies estimating the index case for SARS-CoV-2 having occurred between mid-October to mid-November 2019.[140,141] Using the median commencement date in our model, 12 January 2020 would be day 77 of the outbreak. At this stage, there were 41 hospitalised cases and two deaths that had been reported by Wuhan authorities.[142]

All three metrics are compared as shown in Table 3.5. The population used was 8,266,000 persons, the 2019 population of Wuhan, China.[170] As can be seen, by all three scenario metrics examined, the median number of symptomatic cases that the model predicts falls within the 95%CI range of the Imperial estimate (427-4,471 symptomatic cases by 12 January 2020).[142] All outcome measures in our model are in agreement with what was known at the time about hospitalised cases and deaths, with the one exception that our model estimates that by the time there are two deaths, there have been only 22 (IQR: 11-38) hospitalised cases total, which is lower than the 41 hospitalised cases reported in Wuhan at the time. However, 41 hospitalised cases are still within our full model bounds, which has a range of 2 - 224 hospitalised cases over 100,000 simulations by the time there have been two deaths.

Table 3.5. External comparison of SARS-CoV-2 stochastic model outputs with an estimate by Imperial using three metrics: outbreak day, cumulative number of hospitalised cases and number of deaths known as of 12 January 2020. The cumulative number of symptomatic cases predicted by our model are in **bold,** to compare with the main estimate used in the Imperial study (427-4,471 symptomatic cases by 12 January 2020).[142] IQR: Interquartile Range.

Metric	Day of Outbreak	Hospitalised Cases Total	Deaths	Total Outbreak Size	**Cumulative Symptomatic Cases**
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	**Median (IQR)**
Day 77	-	23 (10 - 43)	1 (0 - 3)	5,007 (2,283 - 6,841)	**1,865 (852 - 3,527)**
41 Cases*	83 (77 - 90)	-	3 (2 - 4)	9,450 (8,471 - 10,497)	**3,522 (3,157 - 3,911)**
2 Deaths	78 (71 - 87)	22 (11 - 38)	-	5,779 (3,312 - 9,297)	**2,153 (1,233 - 3,466)**

* Cumulative number of hospitalised cases, representing the total number of cases known to public health authorities at the time

These results imply that during the early stage of the emergence of a SARS-CoV-2 type of outbreak, the number of total cases likely greatly exceeds the number known in hospitals. This includes both symptomatic and total numbers of outbreak cases. In addition, the outbreak has likely been going on for a longer period of time than initially recognised.

3.4.4 Sensitivity Analysis

A sensitivity analysis was conducted to see how the main results of the time-to-outbreak detection and size at discovery varied based on different disease parameters. First, the parameters were varied to determine the time-to-detection of greater than one hospitalised case, as shown in Figure 3.8. As can be seen, for each parameter, higher values of $\mathcal{R}_0$ decrease the time-to-detection. This is because, with other factors held constant, increasing the $\mathcal{R}_0$ will accelerate the epidemic and decrease the time to first hospital presentation. For a given $\mathcal{R}_0$, the longer the latent or asymptomatic infectious period of a disease, the time to outbreak detection increases. This is also true for greater lengths of time for symptom development or time spent in the symptomatic infectious class. Diseases with a low probability of symptoms and hospitalisation have larger delays in detection time. The length

of time in the hospitalised class and the probability of death do not impact time-to-detection for a given $\mathcal{R}_0$ value when detection is determined by the first hospitalised case.

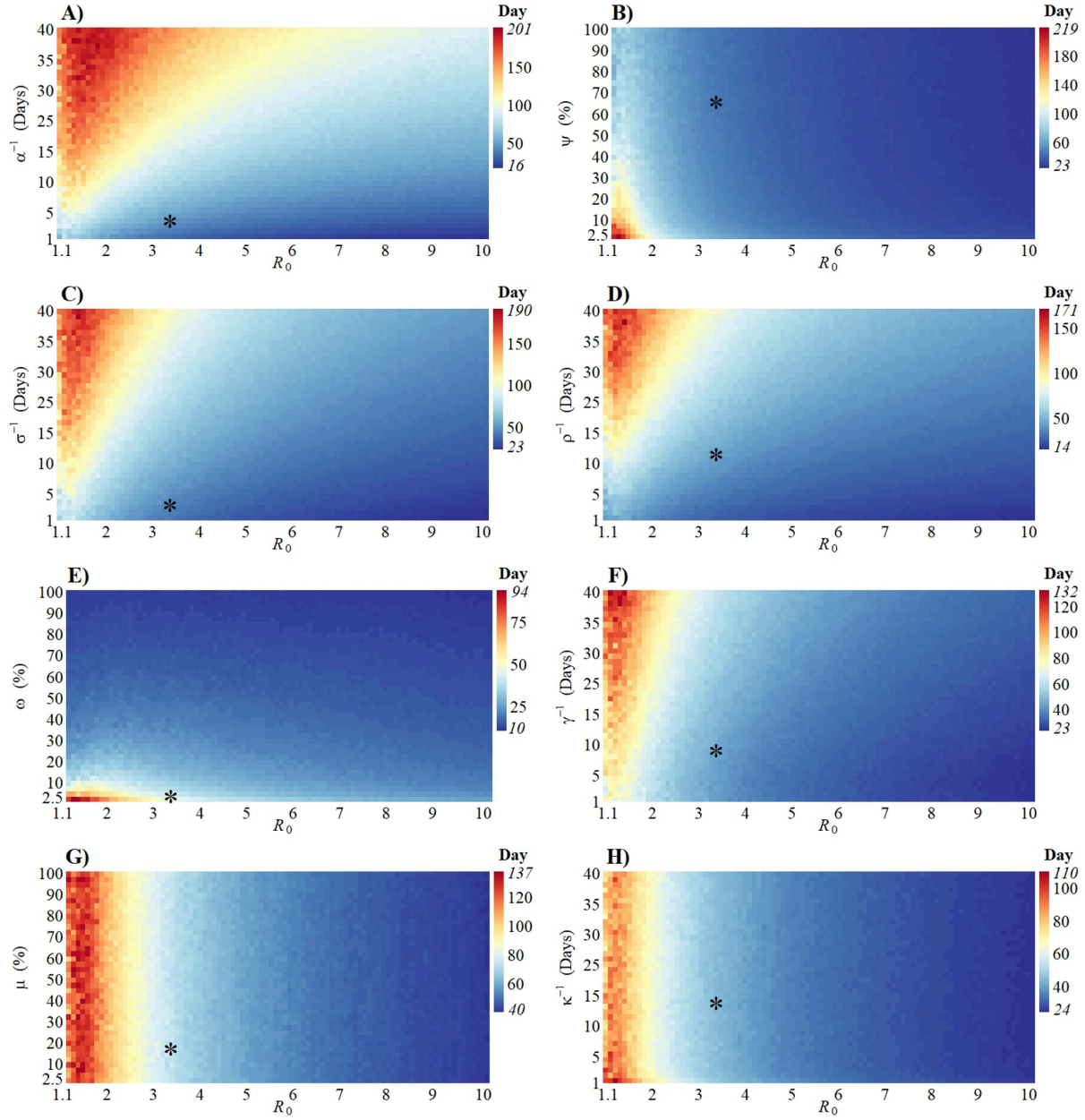


Figure 3.8. Two-way sensitivity analysis heatmaps depicting the number of days to detection through the first case presentation to hospital for eight parameters against different values of $\mathcal{R}_0$. The stochastic model was run for 1,000 simulations for each parameter and $\mathcal{R}_0$ value with other components held constant. Each pixel reflects a given set of parameter values and the colours represent the median time from outbreak commencement to detection through the first hospital presentation for all large outbreaks, with blue representing a shorter detection time and red representing a longer detection time. The asterisks (*) depict the parameter values for the base SARS-CoV-2 model. Italics represent the maximum and minimum detection day. A: The length of the latent period (α^{-1}) in days vs $\mathcal{R}_0$; B: The probability of developing symptoms (ψ) vs $\mathcal{R}_0$; C: Length of time for symptom development (σ^{-1}) in days vs $\mathcal{R}_0$; D: Length of time in symptomatic infectious class (ρ^{-1}) in days vs $\mathcal{R}_0$; E: The probability of hospitalisation given symptoms (ω) vs $\mathcal{R}_0$; F: The asymptomatic infectious period (γ^{-1}) in days vs $\mathcal{R}_0$; G: The probability of death given hospitalisation (μ) vs $\mathcal{R}_0$; H: The length of time in the hospitalisation class (κ^{-1}) in days vs $\mathcal{R}_0$.

The outbreak size at the time of detection is depicted in Figure 3.9 after conducting a $\log_{10}$ transformation of the results. For all eight parameters examined, increasing the $\mathcal{R}_0$ increases the total epidemic size at the point of detection. When combined with the results seen in Figure 3.8, some interesting trends emerge. Given the exponential growth in epidemic size in the early stages, high $\mathcal{R}_0$ outbreaks will lead to earlier detection through accelerating the time to first hospital presentation; however, the outbreak size at discovery will be significantly larger. A pathogen with a longer latent or symptom development period increases the time-to-detection, but the outbreak size at the stage of discovery does not significantly increase. Diseases with a lower probability of symptoms or hospitalisation can grow to large sizes before detection will take place when the threshold requires hospital presentation. The length of time in the hospitalised class and the probability of death do not affect the outbreak size at discovery for a given $\mathcal{R}_0$ value.

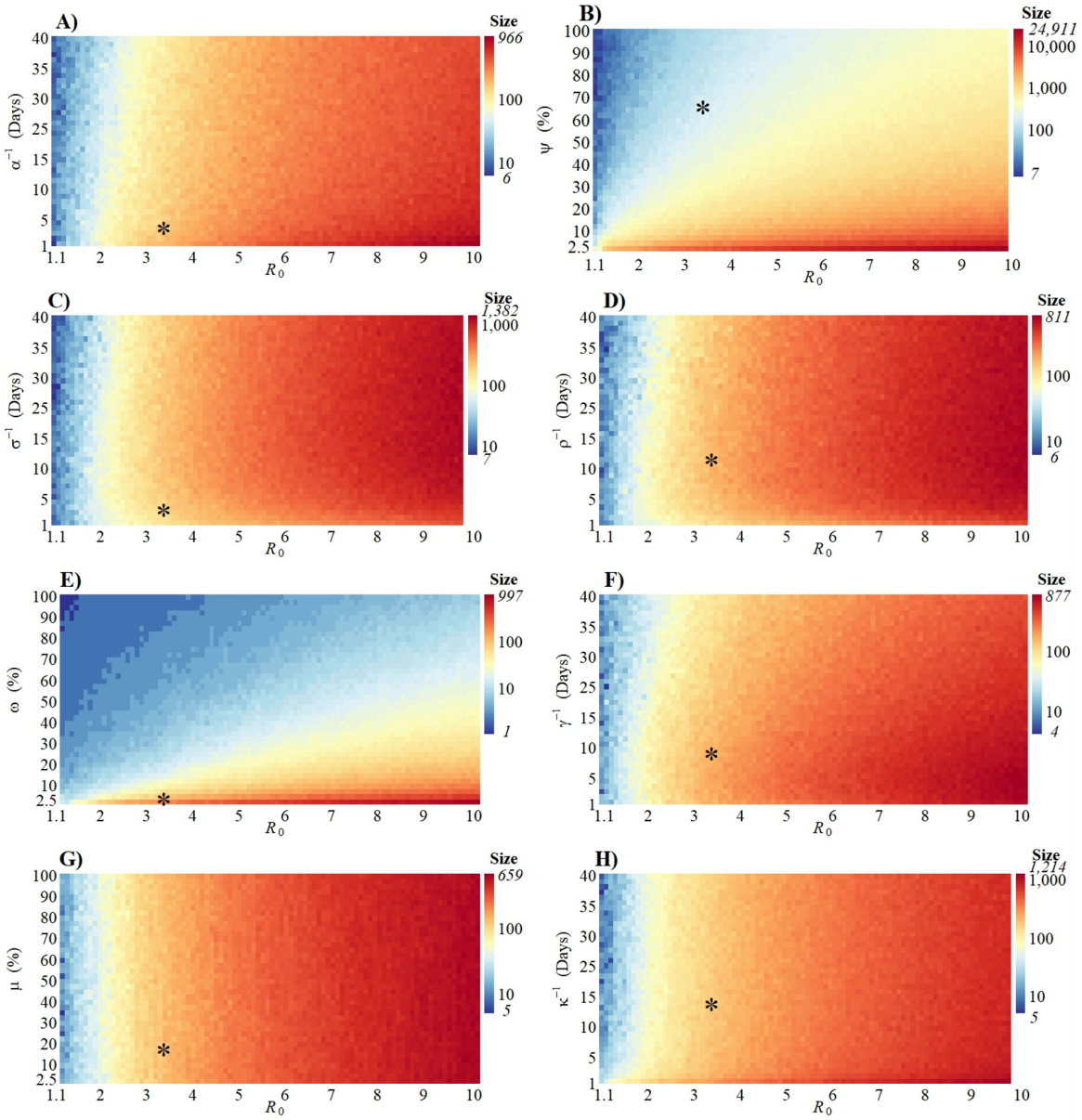


Figure 3.9. Two-way sensitivity analysis heatmaps depicting the $\log_{10}$ transformed size at detection for eight varied parameters against different values of $\mathcal{R}_0$. The stochastic model was run for 1,000 simulations for each parameter and $\mathcal{R}_0$ value with other components held constant. Each pixel reflects a given set of parameter values and the colours represent the median total outbreak size at detection through the first hospital presentation for all large outbreaks, with blue representing a smaller size and red representing a larger size. The asterisks (*) depict the parameter values for the base SARS-CoV-2 model. Italics represent the maximum and minimum size at detection. A: The length of the latent period (α^{-1}) in days vs $\mathcal{R}_0$; B: The probability of developing symptoms (ψ) vs $\mathcal{R}_0$; C: Length of time for symptom development (σ^{-1}) in days vs $\mathcal{R}_0$; D: Length of time in symptomatic infectious class (ρ^{-1}) in days vs $\mathcal{R}_0$; E: The probability of hospitalisation given symptoms (ω) vs $\mathcal{R}_0$; F: The asymptomatic infectious period (γ^{-1}) in days vs $\mathcal{R}_0$; G: The probability of death given hospitalisation (μ) vs $\mathcal{R}_0$; H: The length of time in the hospitalisation class (κ^{-1}) in days vs $\mathcal{R}_0$.

3.4.5 Applying Public Health Interventions

Next, an investigation was undertaken to determine whether isolation of all hospitalised cases (H) or both hospitalised and infectious symptomatic cases (H + I) could control a SARS-CoV-2 outbreak, defined as a $\mathcal{R}_e < 1$. Isolating all hospitalised cases with 100% effectiveness will render $\beta_H = 0$ and produce a $\mathcal{R}_e$ as shown in Equation 3.6. Additionally, isolating all symptomatic cases, such as through public health messaging advising all persons with symptoms to self-isolate, will mean that $\beta_I = 0$ as well, giving the $\mathcal{R}_e$ expression as shown in Equation 3.7. The median day where 50% of all large outbreaks have had at least one hospitalised case was extracted from the stochastic model which was Day 46 since the first index infection. We then initiated the deterministic model with the median values in each SEPIR-AHD compartment derived from the stochastic model from this date onwards.

$$\mathcal{R}_e = \frac{\beta_P \psi}{\sigma} + \frac{\beta_I \psi}{\rho} - \frac{\beta_A (\psi - 1)}{\gamma} \tag{3.6}$$

$$\mathcal{R}_e = \frac{\beta_P \psi}{\sigma} - \frac{\beta_A (\psi - 1)}{\gamma} \tag{3.7}$$

As can be seen in Table 3.6, isolation of only the hospitalised cases has a minimal to no effect on the final epidemic size, peak epidemic size or time to peak, the peak hospital caseload, total deaths or $\mathcal{R}_e$ when compared with no intervention. This is likely due to the small number of hospitalised cases compared to the total number of infectious individuals. Isolating all symptomatic cases, which includes all persons in the I and H compartments, reduces the final epidemic size from 95.7% of the population to 30.5% of the population and significantly reduces the peak size from 36.3% to 2.3%. The time to the peak more than doubles from 114 days to 276 days, and the peak hospital caseload decreases from a maximum of 0.44% of the

population to 0.036% of the population. The total deaths incurred decreased from 0.31% of the population to 0.10% of the population. Although the $\mathcal{R}_e$ reduces from 3.28 to 1.19, it does not fall below the critical value of 1.0, indicating the outbreak will not go extinct with these public health strategies alone, even under the idealised assumption of perfect isolation of all symptomatic and hospitalised cases.

Table 3.6 Deterministic model outputs comparing three public health intervention scenarios.

Public Health Intervention Scenario	Final Epidemic Size*	Peak Epidemic Size*	Time to Peak	Peak Hospital Caseload*	Total Deaths*	Reproductive Number†
	%	%	Days	%	%	$\mathcal{R}_0$ or $\mathcal{R}_e$
1. No intervention	95.7	35.2	113	0.44	0.31	3.28
2. Isolate all hospitalised cases	95.3	34.9	114	0.44	0.31	3.22
3. Isolate all hospitalised and symptomatic cases	30.5	2.3	276	0.036	0.10	1.19

* As a proportion of the total population 100,000 persons
† Basic reproductive number ($\mathcal{R}_0$) for no intervention and effective reproductive number ($\mathcal{R}_e$) for all other scenarios with isolation-based public health interventions. For Scenario 2, the $\mathcal{R}_e$ equation is shown in Equation 3.6 while for scenario 3 the $\mathcal{R}_e$ equation is shown in Equation 3.7.

If we assume that $\beta_P = \beta_A = \beta$ we can determine the amount by which we need to reduce effective contact rate in the presymptomatic and asymptomatic populations in order for the reproductive number to fall $\mathcal{R}_e < 1$ in the scenario in which all symptomatic cases are isolated. First, Equation 3.7 can be rearranged, and then solved for β as shown in Equation 3.8.

$$\mathcal{R}_e = \beta\left(\frac{\psi}{\sigma} - \frac{(\psi-1)}{\gamma}\right)$$

$$\beta < \left(\frac{\psi}{\sigma} - \frac{(\psi-1)}{\gamma}\right)^{-1} \tag{3.8}$$

Using the SARS-CoV-2 parameter values presented in Table 3.3, this implies that β needs to be reduced from 0.24 to less than 0.20 for the outbreak to die off even when perfectly isolating all symptomatic and hospitalised cases. Such a decrease in the effective contact rate would need to be achieved through additional public health intervention strategies that target asymptomatic and pre-symptomatic cases, such as contact tracing with quarantine, social distancing mandates, or population-level lockdowns.

3.4.6 Surveillance Coverage Strategies

Different surveillance strategies were compared to determine the coverage level required to detect the outbreak at different time points and total sizes. Figure 3.10 depicts six surveillance coverage levels against the detection threshold of ≥ one case in hospital. If surveillance coverage is 0.1% of the population, the median (IQR) time-to-detection is 43 (35 - 49) days, while at 1% this decreases to 23 (16 - 29) days. In order to detect 50% of SARS-CoV-2 outbreaks at a time point at or before the first case presents to a hospital, the minimum surveillance level required is 0.069% (IQR: 0.028 - 0.139%). This means in a population of 100,000 that 69 people (IQR: 28 - 139) need to be tested per day at random in order to detect the outbreak before the median time point at which detection occurs with one or more people in hospital. To detect 90% of outbreaks before the first presentation, this increases to 235 people per day in a population of 100,000.

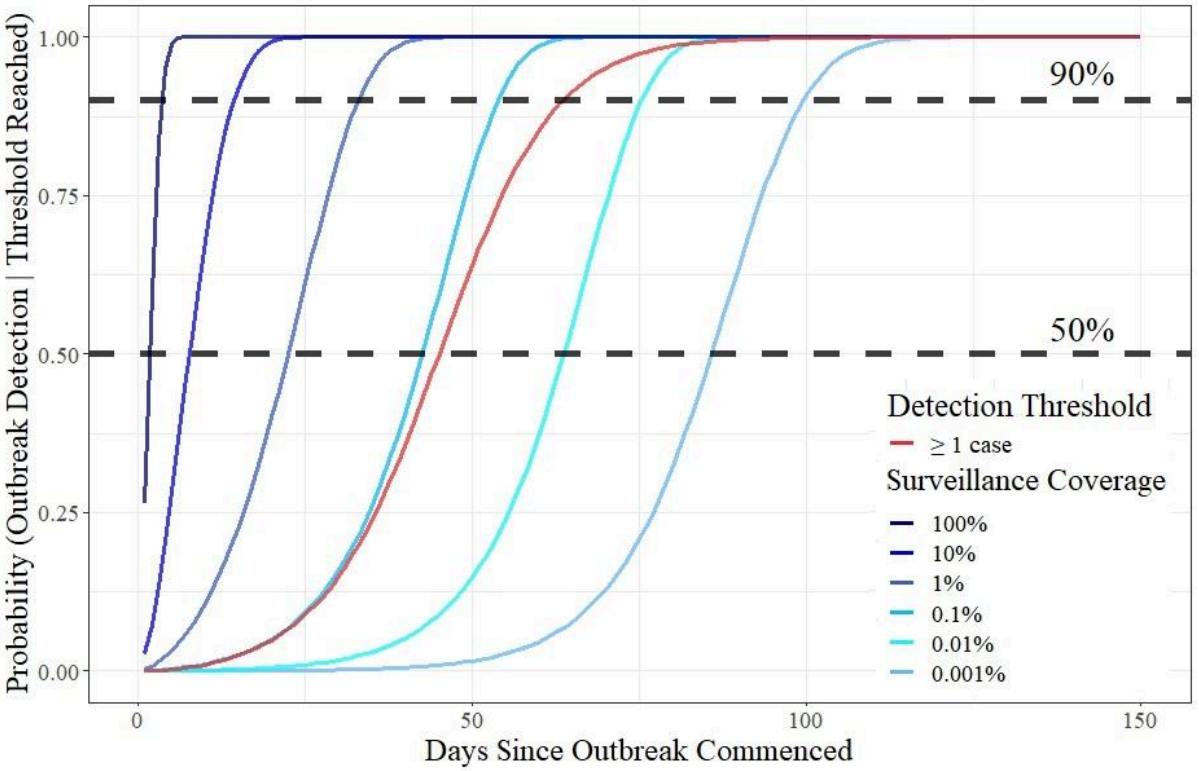


Figure 3.10 Probability of SARS-CoV-2 outbreak detection vs epidemic day for all large outbreaks comparing the detection threshold of ≥ one case in hospital to six different surveillance coverage levels on a $\log_{10}$ scale from 0.001% to 100%. The black dotted lines represent when 50% and 90% of outbreaks will have been detected on a given day.

In contrast with the above results, to detect 50% of outbreaks by the time there are four hospitalised cases, as was the case at the time of first detection of SARS-CoV-2 in December 2019, 0.01% (IQR: 0.004 - 0.02%) of the population need to be tested per day. In a population of 100,000 people, this equates to 10 people tested per day. To detect 90% of outbreaks before the time there are four hospitalised cases, 0.034% need to be tested per day, or 34 out of a 100,000 person population.

In Figure 3.11, the probability of outbreak detection for a given total outbreak size is compared for all large outbreaks. As can be seen, when the total outbreak size is $\log_{10}$ transformed, the plot follows a trajectory similar to what was seen in Figure 3.10. At smaller outbreak sizes, there is greater stochastic noise. At 0.1% surveillance coverage, the median total size when 50% of the outbreaks will have been detected was 124 (IQR: 52 - 238)

cumulative infections. In contrast, with 1% surveillance coverage, 50% of outbreaks will be detected by 14 (6 - 27) total infections.

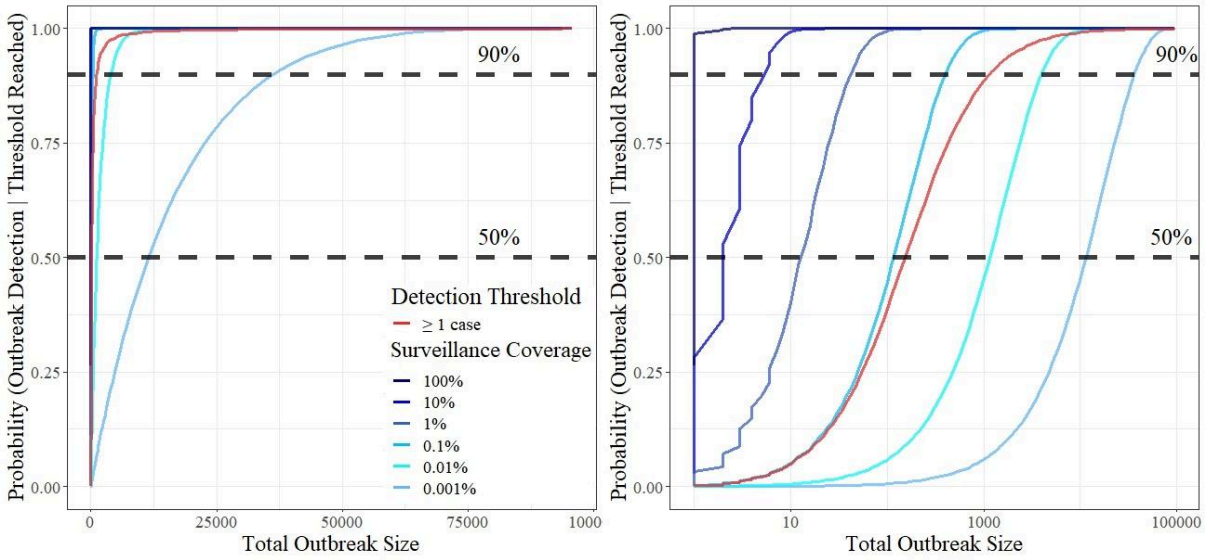


Figure 3.11 Probability of outbreak detection over the median total outbreak size for all large outbreaks comparing the detection threshold of ≥ one case in hospital to six different surveillance coverage levels on a $\log_{10}$ scale from 0.001% to 100%. The black dotted lines represent when 50% and 90% of outbreaks will have been detected at a given total outbreak size. A) Original unscaled plot. B) Log_{10} transformed x-axis total outbreak size.

In Figure 3.12, the median day of outbreak detection is shown for different levels of surveillance coverage. For comparison, the median day of outbreak detection using traditional hospital-based surveillance is included with the detection threshold set to ≥ one case in the hospital, or day 46. As can be seen, the surveillance coverage level required to detect the outbreak follows an exponential decay trajectory. When plotted against outbreak detection size, as shown in Figure 3.13, outbreak detection follows a power law distribution and is approximated to be linear on a log-log plot.

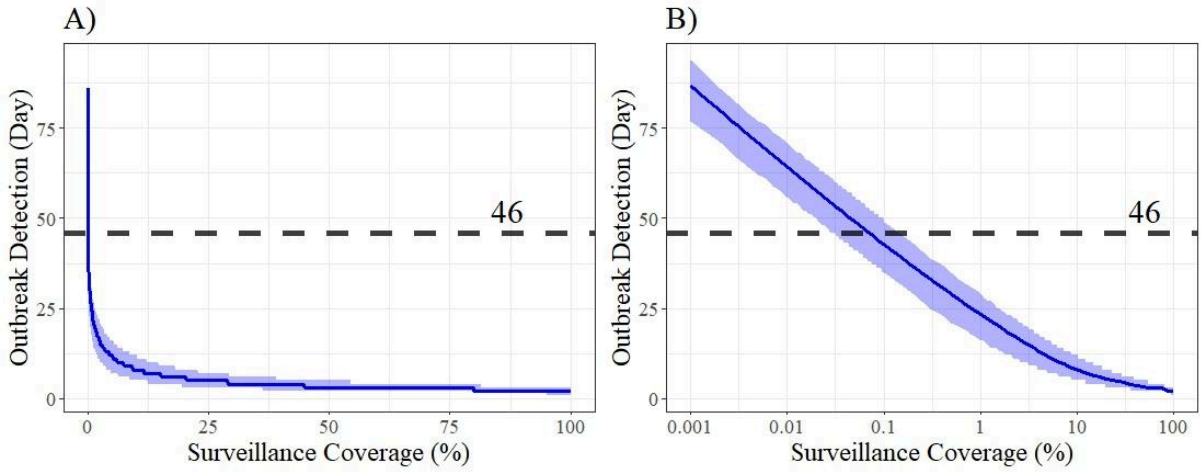


Figure 3.12 Day of outbreak detection vs surveillance coverage level. The solid blue line represents the median value with the IQR in shaded blue. The black dotted line represents the median detection day using the detection threshold of ≥ one case in hospital (day 46). A) Non-transformed data. B) Log_{10} transformed x-axis surveillance coverage.

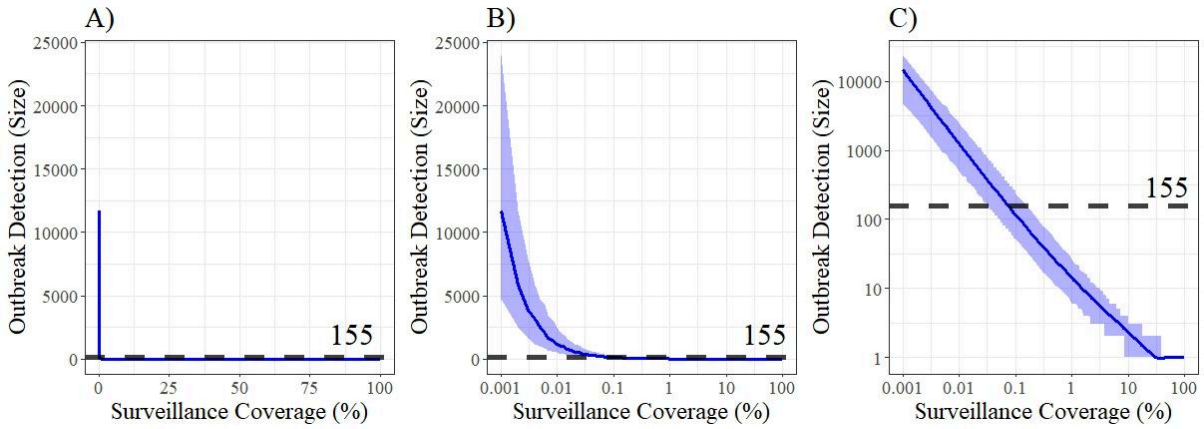


Figure 3.13 Outbreak size at detection vs surveillance coverage level. The solid line represents the median value with the IQR in shaded blue. The black dotted line represents the median detection size using the detection threshold of ≥ one case in hospital (155 total infections). A) Non-transformed data. B) Log_{10} transformed x-axis surveillance coverage. C) Log-log transformation of both surveillance coverage and outbreak size.

To generalise the results beyond the transmission dynamics of SARS-CoV-2 infections, heatmaps of variable parameters against $\mathcal{R}_0$ are depicted in Figure 3.14. Each pixel represents the surveillance coverage levels needed to detect the outbreak on or before the first case presents to the hospital, for random testing conducted daily in the population without replacement. Generally, the higher the $\mathcal{R}_0$, the lower the coverage level required to detect the disease. Changes in the latent period had a mild impact on the surveillance level needed. In contrast, changes in symptom and hospitalisation probability had a significant impact, with

higher values requiring larger numbers of tests each day. Changes to the length of time in the hospitalisation class and probability of death did not significantly change the coverage level required for a given $\mathcal{R}_0$ value.

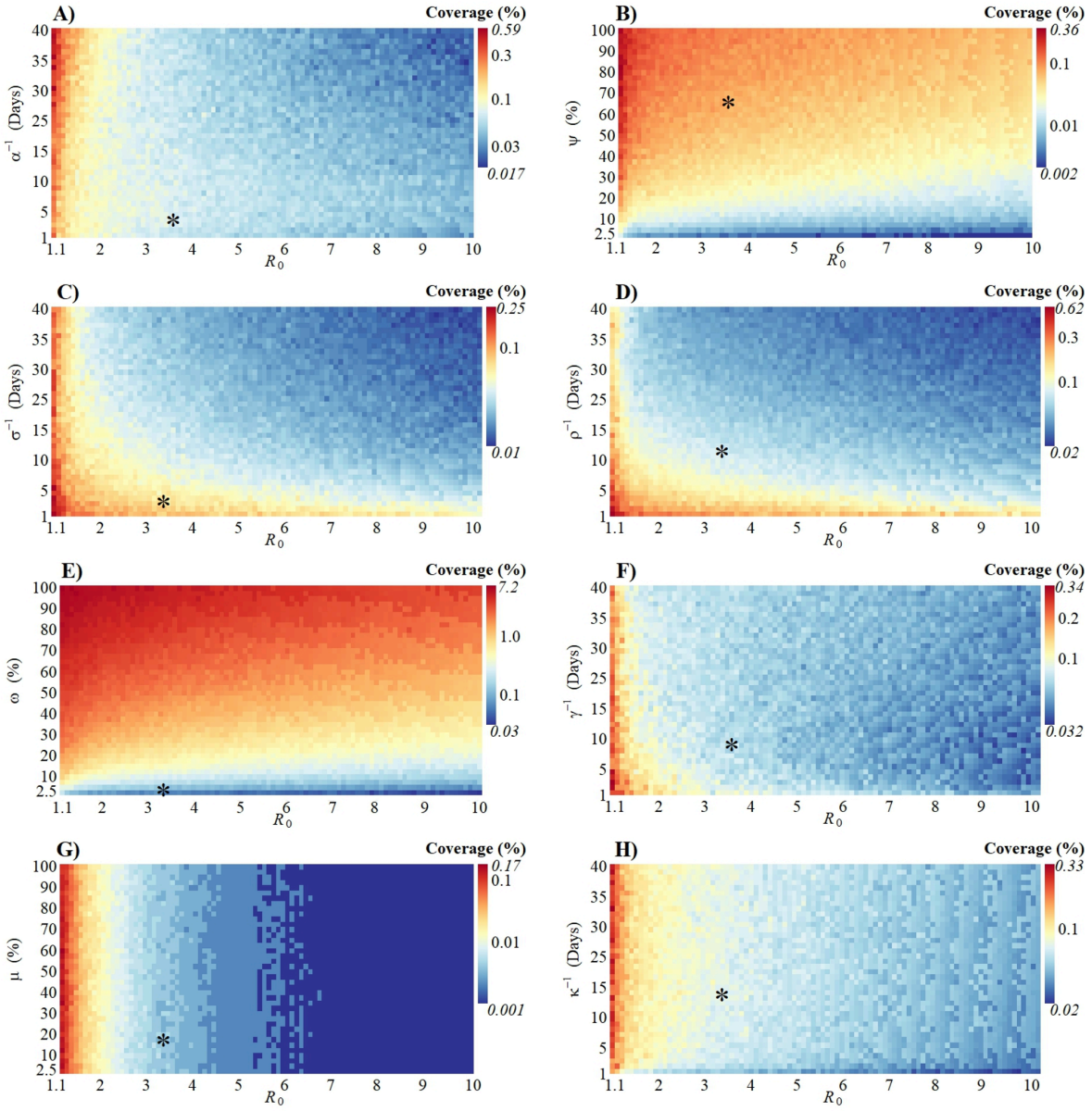


Figure 3.14 Two-way sensitivity analysis heatmaps depicting the $\log_{10}$ transformed minimum coverage level required to detect the outbreak before the first case presentation to hospital for eight varied parameters against different values of $\mathcal{R}_0$. The stochastic model was run for 1,000 simulations for each parameter and $\mathcal{R}_0$ value with other components held constant. Each pixel reflects a given set of parameter values and the colours represent the minimum surveillance coverage level needed to detect the outbreak, with blue representing a smaller coverage level needed and red indicating a larger coverage requirement. The asterisks (*) depict the parameter values for the base SARS-CoV-2 model. Italics represent the maximum and minimum coverage level. A: The length of the latent period (α^{-1}) in days vs $\mathcal{R}_0$; B: The probability of developing symptoms (ψ) vs $\mathcal{R}_0$; C: Length of time for symptom development (σ^{-1}) in days vs $\mathcal{R}_0$; D: Length of time in symptomatic infectious class (ρ^{-1}) in days vs $\mathcal{R}_0$; E: The probability of hospitalisation given symptoms (ω) vs $\mathcal{R}_0$; F: The asymptomatic infectious period (γ^{-1}) in days vs $\mathcal{R}_0$; G: The probability of death given hospitalisation (μ) vs $\mathcal{R}_0$; H: The length of time in the hospitalisation class (κ^{-1}) in days vs $\mathcal{R}_0$.

Finally, the surveillance coverage levels needed to detect the outbreak at 28 days or earlier were derived for different parameter values. This was completed because a specific target time may be desired by a public health system instead of surpassing the time-to-detection through surveillance which relies on hospital presentations. The results are shown in Figure 3.15. As can be seen, diseases with longer latent periods need greater surveillance coverage, with requirements up to 8.81% of the population for a latent period of 40 days. Increasing the symptom and hospital probability had a small but notable effect on increasing the coverage levels required. This is because, in the model, this increases the proportion of individuals who proceed into the symptomatic and hospitalised classes and thus results in a larger force of infection as defined in Equation 3.1 for a given value of β, derived from a given value of $\mathcal{R}_0$.

When Figure 3.15 is compared with Figure 3.14, some interesting findings emerge. When the target for detecting the outbreak is based on discovery before the first case presentation to hospital, the $\mathcal{R}_0$ value has a greater effect than the length of the latent period or asymptomatic infectious period; however, when the target is 28 days, the opposite is true. To discover the outbreak before the first case in hospital, the symptom and hospitalisation probabilities alter the surveillance coverage levels needed more than the $\mathcal{R}_0$ value, but when the target is 28 days, the $\mathcal{R}_0$ value has a greater impact. For both types of surveillance targets, increasing the length of time in the hospitalisation class and probability of death did not significantly change the coverage level required for a given $\mathcal{R}_0$ value.

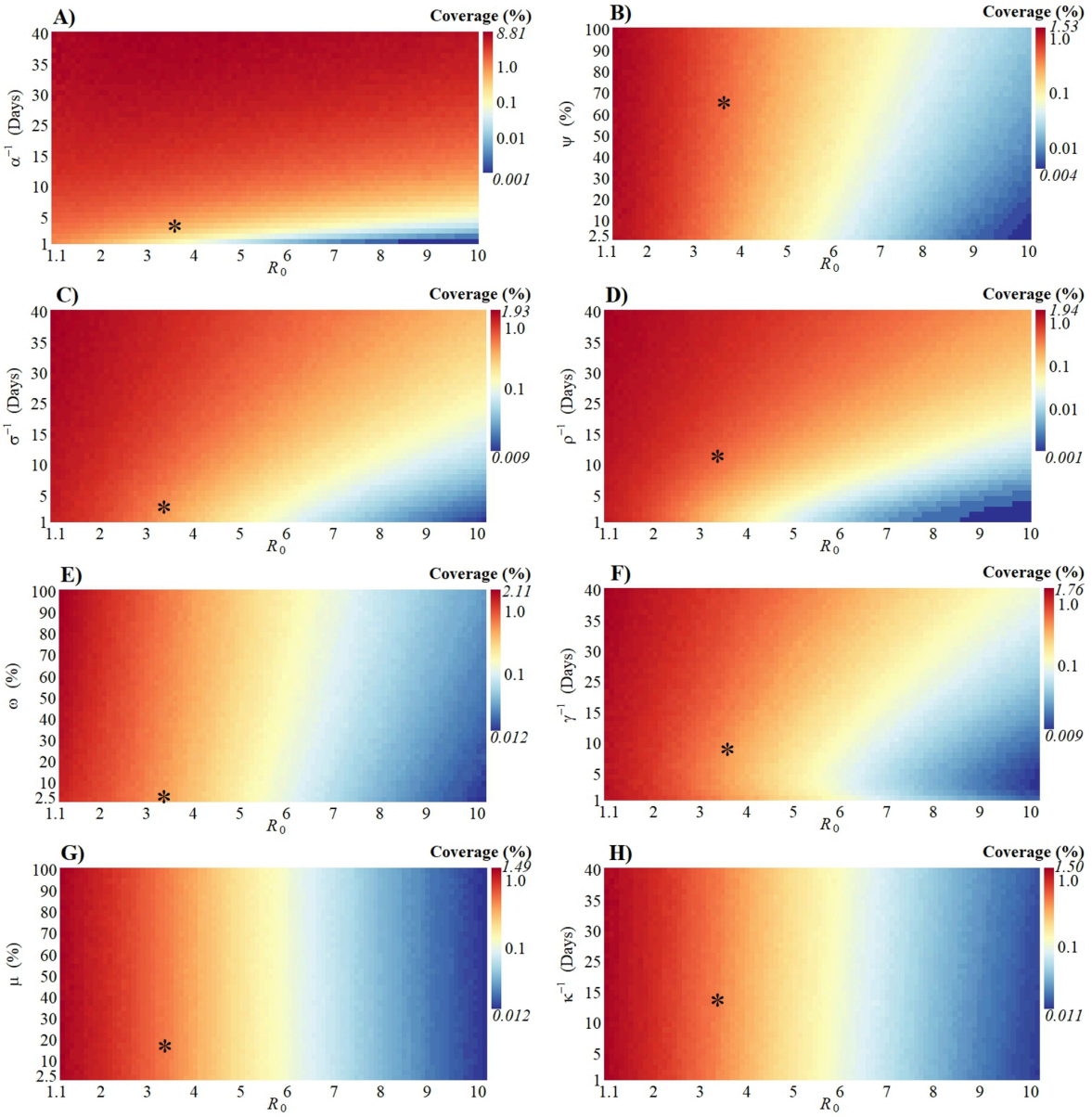


Figure 3.15. Two-way sensitivity analysis heatmaps depicting the $\log_{10}$ transformed minimum coverage level required to detect the outbreak within the first 28 days for eight varied parameters against different values of $\mathcal{R}_0$. The stochastic model was run for 1,000 simulations for each parameter and $\mathcal{R}_0$ value with other components held constant. Each pixel reflects a given set of parameter values and the colours represent the minimum surveillance coverage level needed to detect the outbreak, with blue representing a smaller coverage level needed and red indicating a larger coverage requirement. The asterisks (*) depict the parameter values for the base SARS-CoV-2 model. Italics represent the maximum and minimum coverage level. A: The length of the latent period (α^{-1}) in days vs $\mathcal{R}_0$; B: The probability of developing symptoms (ψ) vs $\mathcal{R}_0$; C: Length of time for symptom development (σ^{-1}) in days vs $\mathcal{R}_0$; D: Length of time in symptomatic infectious class (ρ^{-1}) in days vs $\mathcal{R}_0$; E: The probability of hospitalisation given symptoms (ω) vs $\mathcal{R}_0$; F: The asymptomatic infectious period (γ^{-1}) in days vs $\mathcal{R}_0$; G: The probability of death given hospitalisation (μ) vs $\mathcal{R}_0$; H: The length of time in the hospitalisation class (κ^{-1}) in days vs $\mathcal{R}_0$.

For SARS-CoV-2, to detect 50% of outbreaks at 28 days or earlier, at least 0.51% (IQR: 0.21 - 1.04%) of the population need to be tested at random each day. This equates to 511 or more

people being randomly tested daily in a population of 100,000. In order to detect 90% of outbreaks by 28 days or earlier, 1,752 people need to be randomly tested every day, or 1.75% of the population. If we decrease the time target to 14 days, 50% of outbreaks will be detected if we conduct surveillance testing on at least 3.13% (IQR: 1.30 - 6.28%) of the population per day. We can detect 90% of outbreaks by day 14 if 10.49% of the population is tested at random every day. If the time target is 7 days, 50% of outbreaks will be detected by this time point if 11.66% (IQR: 4.90 - 22.81%) of the population is tested per day, with 90% detected if 36.71% of the population is tested per day.

Section 3.5 Discussion

Our study investigated novel pathogen outbreak detection and surveillance using a range of modelling techniques. We employed a stochastic SEPIR-AHD model, initially parameterised to SARS-CoV-2 transmission dynamics, and explored the probability of outbreak discovery for different thresholds utilised by countries around the world. Our results focused on two key public health metrics: the time-to-detection and total outbreak size at discovery. After externally comparing our model to an early estimate in the literature and investigating the effect of potential public health interventions, we explored how our results change across a range of disease parameters. Finally, we analysed random testing in the population to determine the surveillance coverage levels needed to detect a novel pathogen outbreak before the first case presentation to hospital, and within seven, 14 or 28 days of the first exposure.

Our results show that while almost all SARS-CoV-2 outbreaks which grow large enough to have at least one hospitalised case go on to have five or more hospitalised cases and two or more deaths, countries with larger detection thresholds will have an increased delay to detection and greater outbreak size at discovery. This provides an imperative for countries to

aim for detection at the first case presentation to hospital rather than other thresholds, given that for outbreaks of novel pathogens like SARS-CoV-2, by the time this criterion is met, it is unlikely the outbreak will go extinct without public health interventions. Mitigation strategies will also be more likely to succeed if the outbreak is detected at an earlier stage.[42]

In our SARS-CoV-2 model, 50% of large outbreaks will have one or more hospitalised cases by day 46, at which stage 155 individuals will have been infected with the virus. If outbreak detection does not take place until there are ≥ four cases in hospital, as was the case with COVID-19, our model shows that 50% of outbreaks are detected by day 64, at which stage the outbreak has already infected 1,253 individuals. If this detection threshold is used, this implies SARS-CoV-2 style outbreaks will likely have a larger than two-month delay between outbreak commencement and discovery, and a greater than 300-fold increase in the number of total infections compared with the known cases at time of detection.

Our findings underscore that in most novel pathogen outbreak scenarios similar to the first emergence of SARS-CoV-2, the actual outbreak size is notably larger than the number of known cases if the only infections visible to public health authorities are symptomatic hospitalised cases and deaths. In addition, our model results suggest that relying solely on symptomatic presentation to healthcare facilities for detection can considerably delay the identification of novel pathogen outbreaks. Our model outputs align with an early estimate of the total number of symptomatic cases by Imperial College London which employed a different strategy to estimate the outbreak size, reinforcing the confidence in our results.[142]

Through conducting a sensitivity analysis where model parameters were varied, we demonstrated that diseases with a high $\mathcal{R}_0$ will result in earlier detection. This makes sense

because, for a given set of parameter values, increasing $\mathcal{R}_0$ will increase the number of people infected at each generation and accelerate the epidemic, decreasing the time to the first presentation at healthcare facilities and therefore detection. However, we demonstrate that this is also accompanied by an increase in epidemic size at the point of discovery, meaning that despite being discovered earlier, high $\mathcal{R}_0$ outbreaks will be less containable even with immediate response actions undertaken. Diseases with a longer latent, symptomatic and asymptomatic infectious period and will have a greater time-to-detection but will not necessarily be greater in size at outbreak discovery for a given value of $\mathcal{R}_0$. Infections with lower symptom or hospitalisation presentation probability will have both a delayed detection and larger outbreak size at discovery because detection using current surveillance relies upon healthcare presentation, which in turn relies on the values of these two parameters.

Our results showed that for outbreaks with SARS-CoV-2 transmission dynamics, even with perfect isolation of hospitalised cases or all symptomatic cases, the $\mathcal{R}_e$ will not fall below the critical value of one. Isolating symptomatic individuals, however, can reduce the final epidemic size and peak hospital caseload, potentially preventing health system overwhelm and allowing time for other interventions to be implemented. This finding has significance for public health planning, implying that additional intervention strategies will need to be deployed to curtail epidemic growth. This is because the outbreak can continue to propagate through asymptomatic and pre-symptomatic transmission alone. Indeed, for the COVID-19 pandemic, population-level lockdowns were imposed in multiple countries to control transmission for this reason.[185]

These results highlight the need for additional surveillance strategies to detect novel pathogen outbreaks. If the outbreak is discovered early at a smaller size, local population restrictions

could be sufficient to contain the outbreak.[186,187] Our analysis of surveillance coverage provides valuable insights into the levels of random testing required for early outbreak detection which could allow for timely public health interventions. To detect 50% of SARS-CoV-2 outbreaks before the first case presents to a hospital, 0.069% of the population needs to be tested daily. When generalising the results for various disease parameters, we find that diseases with higher $\mathcal{R}_0$ values require lower surveillance coverage levels for early detection before the first case presentation to hospital. Greater levels of testing would be required for diseases with a high symptom or hospitalisation probability.

If public health authorities aim to detect a novel pathogen outbreak within a certain time limit, such as within 28 days of the first case exposure, the level of surveillance coverage would be significantly greater. To detect 50% of SARS-CoV-2 outbreaks within 28 days, at least 0.51% of the population need to be tested at random each day. The coverage level required would be significantly higher for diseases with a longer latent period. This may not be feasible if conducted through individual testing in the population. However, there have been recent advances in wastewater detection of outbreaks.[188,189] Surveillance over a large portion of the population may be achievable through this form of sampling, as samples are essentially pooled from a given population in wastewater. However, more research needs to be done to determine if pathogen-agnostic sequencing could be sensitive enough to classify genomic reads from this form of testing.[190] At very low prevalence levels, as would be the case in the early stages of a novel disease outbreak, it is unclear whether metagenomic sequencing would be able to detect a new pathogen signal through the background noise inherent to this type of sample. Modifying the model to account for test sensitivity and detection through wastewater could offer valuable insights.

While some novel diseases may be detectable through population-level surveillance, those with a long latent period may prove the most challenging given high levels of coverage required to detect within a given timeframe. However, while detection may be delayed, our results show that the outbreak size at discovery would not necessarily increase with prolonged latent periods, given a specific $\mathcal{R}_0$ value. This means that by the time it is eventually detected, diseases with this characteristic may still be discovered in time for public health interventions to have a positive effect on reducing morbidity and mortality. However, delays to detection also allow for spread to new geographical locations, and thus can complicate the public health response. This information can help public health authorities design surveillance strategies that consider differing characteristics of emerging pathogens. In addition, our results demonstrated significant differences in the parameter effects on the coverage levels required if the surveillance target is based on detection of the outbreak before the first case presentation to hospital or within a specified time target. For example, the length of the latent period had a greater impact than the $\mathcal{R}_0$ value on coverage levels when the target was 28 days, while the opposite was true if the target was to discover the outbreak before the first hospital presentation. These differences are important to consider when choosing between different public health targets for surveillance system design.

3.5.1 Strengths and Limitations

This study benefited from the ability to model a novel pathogen outbreak based on a real-world event of a new disease emergence with SARS-CoV-2 parameters published in the literature. We showed that our results are robust upon external comparison with research using a different methodological approach, increasing confidence in our model design. Our work investigated novel approaches to public health surveillance, providing a useful starting point to examine potential applications of population-level testing using pathogen-agnostic

detection for future outbreaks. Our model results provide a preliminary set of population coverage targets that would need to be achieved under idealised conditions, and these could be investigated through a future cost-benefit analysis to assess feasibility.

There are some limitations to this study. First, the model assumes that the outbreak begins with a single exposed individual and does not consider scenarios such as simultaneous multiple exposures, which could lead to different results through an accelerated early stage of the outbreak. Second, the model does not consider the potential impact of different effective contact rates for the four infected classes. Thirdly, the model does not account for test sensitivity and the cost of implementing background population surveillance, factors highly important for the design of a new detection system for novel diseases. Lastly, our model was designed based on SARS-CoV-2, and while our two-way sensitivity analyses offer insights into how variations in the parameters change the outputs of the model, the results obtained are not fully applicable to some diseases without a change in model structure or varying greater than two parameter values simultaneously.

The current model operates under the assumption hospitalisation or death from a new disease is not missed and detected without delay. In reality, some presentations of a new disease may be missed (e.g., classified as viral pneumonia without definitive diagnosis) and the ones that are not will likely have a reporting delay, such as through waiting on laboratory processing of results. We partially address this by including the detection threshold of four or more hospitalised cases in our model, to represent the scenario faced during the discovery of the first SARS-CoV-2 cases. Our model does not account for various factors that could influence the detection of a new disease, such as unusual or unique symptoms, mimicry with other

diseases, and discovery through EBS systems. These factors could significantly impact the sensitivity and timeliness of novel disease detection.

3.5.2 Future Research Directions

Previous work has highlighted the importance of three factors for early detection: population coverage, sampling frequency and test sensitivity.[165] Our work has focused on population coverage, while previous research has been completed on the importance of sampling frequency.[137] Future work could focus on incorporating test sensitivity into our detection model, accounting for the potential of false negatives during surveillance. This will require further empirical research into the sensitivity of mNGS-based detection for novel pathogens. Determining detection specificity would also be useful to estimate how often a new surveillance system may need to respond to false positive outbreak signals, which could impact resource requirements and implementation cost.

We varied our model parameters against a range of $\mathcal{R}_0$ values, but given a large number of degrees of freedom when it comes to transmission dynamics between diseases, future research could investigate the importance of other parameter comparisons, such as between the latent period length and hospitalisation probability. Re-parameterisation of the model for other known infectious diseases could also lead to further insights, although this may require different model structures (such as removal of the pre-symptomatic compartment) depending on the pathogen transmission dynamics in question.

We could also expand our model into a more comprehensive network system. This would allow us to assess the potential outbreak size by the time a case has spread between jurisdictions with different novel disease detection thresholds. By simulating the spread of

emerging infectious diseases within and across countries, the network model could help to identify whether detection is most likely to occur in locations beyond the point of origin. This could also lead to insights into whether early warning systems with novel disease detection capabilities should be prioritised for certain hotspot regions. Future work could identify major network nodes — such as urban centres, international transport hubs, or regions with dense inter-jurisdictional movement — that may act as pivotal points of disease spread. Strengthening novel pathogen detection capabilities at these nodes could serve as a robust and strategic defence against the international proliferation of infections if combined with a rapid public health response. Furthermore, understanding these critical nodes would allow policymakers and health authorities to prioritise the allocation of limited resources, ensuring that early detection and intervention mechanisms are optimally placed. This could lead to insights into whether early warning systems with novel disease detection capabilities should be prioritised for certain hotspot regions.

To improve the accuracy of our model, future research could consider more realistic scenarios for the initial hospitalisation and detection of new diseases. For example, incorporating the likelihood of missed cases and the time delay between presentation to the health service and definitive diagnosis could provide a more accurate representation of real-world situations. By simulating these scenarios, we could identify other potential bottlenecks in the detection process and develop strategies to address them, ultimately improving the overall effectiveness of novel disease detection systems.

3.5.3 Conclusion

This Chapter highlights the importance of early detection for novel pathogen outbreaks, demonstrating the potential delays and size at the point of discovery. By understanding some

of the dynamics of outbreak detection and the impact of different disease parameters on surveillance coverage levels, public health authorities can build on our model to further understand the costs and benefits of population-level surveillance. With the potential impact of novel diseases on public health and global economies, developing and optimising effective detection strategies is of great importance. Recognising that early detection is vital for limiting the spread and impact of emerging infectious diseases, we emphasise the importance of assessing the feasibility and continuous refinement of early warning systems. Our research offers a preliminary exploration of the role of novel disease surveillance in the early identification of emerging infectious diseases. However, it will be crucial in future work to incorporate additional factors such as test sensitivity and implementation cost to improve understanding and address the complexities of novel disease detection.

3.5.4 Data Availability

The model used in this Chapter is available for download through the Open Science Framework platform, along with all code used to generate the results, tables and figures. ❄

4

Community Detection and Prevalence of SARS-CoV-2 Infection

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Section 4.1 Abstract

In an outbreak of a novel infectious disease, determining the community prevalence is crucial to guide the public health response and monitor the impact of population-level interventions. This Chapter presents a study conducted to determine the infection prevalence of the novel pathogen Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) during the early stages of the pandemic in the United Kingdom (UK) in the adult Oxfordshire population.

Using a cross-sectional observational study design, 2,908 participants were sampled over five time points between 20 April - 17 June 2020 during the first national UK lockdown. Participants were sent kits to self-collect swabs for reverse-transcription polymerase chain reaction (RT-PCR), and metagenomic next-generation sequencing (mNGS) tests on the same day as completing lateral flow serology tests and a questionnaire which captured data on Coronavirus Disease 2019 (COVID-19) symptoms, risk factors and health behaviours. Three alternative detection methods (mNGS, serology and the symptom-based case definition) were compared against RT-PCR as the gold standard.

This study found the community prevalence of SARS-CoV-2 infection with a 95% binomial confidence interval to be 0.57% (0.16 - 1.45%) on 20 April, 0.38% (0.046 - 1.37%) on 4 May, 0.0% (0.0 - 0.064%) on 18 May, 0.0% (0.0 - 0.85%) on 2 June, and 0.0% (0.0 - 0.81%) on 17 June 2020. These results provided early evidence of community prevalence of SARS-CoV-2 infection in the UK and were consistent with Office for National Statistics (ONS) COVID-19 Infection Survey model estimates. The symptom-based case definition and serology tests were significantly associated with RT-PCR positivity (*p*-values: 0.011 and 0.0052, respectively) and had high diagnostic odds ratios. This study was underpowered to detect an association between mNGS results and SARS-CoV-2 infection-induced changes in the oropharyngeal microbiomes of participants. Compliance with health behaviours, including social distancing, social isolation, hand washing and keeping a two-metre distance from others, had a decreasing trend over the study period.

Overall, this research showed that the community prevalence of SARS-CoV-2 infection was very low and decreasing in April - June 2020 in Oxfordshire, UK and the majority of RT-PCR positive cases were asymptomatic at the time of testing.

Section 4.2 Introduction

In the early stages of a novel pathogen outbreak, the true prevalence of infection in the population will often be unknown. Case finding is an essential component of epidemic investigations and necessary for situational awareness and strategic decision-making in the public health response.[36] Traditionally, case detection relies upon reporting by medical practitioners and laboratories and will primarily be limited to symptomatic individuals who present to a healthcare facility, undergo assessment, and meet pre-established diagnostic criteria. This surveillance strategy can identify illness in the most clinically important settings

but limits understanding of the disease burden in the wider population. Patients admitted to healthcare facilities may have differences in disease severity and risk factors compared to the background community. Relying on their data alone can incur sampling bias and result in a skewed prevalence estimate that undercounts mild cases.[191]

In a novel pathogen outbreak with unreliable case numbers, researchers may use death reports instead to reduce bias.[192] However, mortality data introduces a time lag and can also vary in completeness. Another strategy to reduce sampling bias is contact tracing, which involves identifying all individuals who have been in close proximity to infected persons. Contact tracing can assist with case detection and provide direct estimates of mild and asymptomatic infections.[147] However, contact tracing has limitations in several outbreak scenarios, especially if some chains of transmission are undetected.[193] In addition, during the exponential growth phase of an outbreak, public health resources can quickly become overwhelmed, rendering contact tracing ineffective.[194]

A population-based study incurs less bias and may be better than traditional surveillance methods to estimate the community prevalence of a new disease accurately.[195] This is particularly important when there is a significant portion of asymptomatic infections. If conducted longitudinally using a representative sample, transmission and risk factors can be monitored in the target population over time. Obtaining prevalence estimates can help health systems predict patient load requirements and ascertain risk factors associated with the disease. For new pathogens that induce a long-term humoral immunity response, community surveillance can assist in modelling background population resistance to the disease and the likelihood of epidemic resurgence. Knowing how prevalence changes over time is also necessary to assess the effectiveness of any population-level public health interventions.

4.2.1 COVID-19 in the United Kingdom

The first known case of COVID-19 in the UK was imported on 23 January 2020 from Hubei Province, China.[196] During the first few months, testing capacity for SARS-CoV-2 was severely limited, leading to the National Health Service (NHS) prioritising tests for hospitalised patients admitted to intensive care units with symptoms of COVID-19.[197] Triaging limited testing resources was a significant public health policy to ensure critically ill patients received confirmed diagnoses. However, this meant that the extent of community transmission of SARS-CoV-2 was unknown in the early stages of the pandemic. One report from 28 March 2020 estimated the mean percentage of the UK population infected to date to be 2.7% with a 95% confidence interval of 1.2 - 5.4%.[198] This estimate is for the cumulative incidence since the outbreak began; the population prevalence in March 2020 was unknown because of the absence of community testing. The situation was rapidly changing, with a doubling time estimate on 23 March 2020 of 2.8 days with a 95% credible interval (CrI) of 2.6 – 3.0 days.[199] At this time, the first national UK lockdown was announced, with stay-at-home orders legally coming into force a few days later.[200]

4.2.2 Detection Approaches

Various detection methods for SARS-CoV-2 infection were used to define cases in the early stages of the COVID-19 pandemic. One of the first public health tasks in a novel pathogen outbreak is establishing a case definition, the criteria by which a person can be assessed to determine whether or not they have the disease.[36] Case definitions can incorporate symptoms, exposure history and test results and need to be sensitive enough to detect true instances of disease while minimising false positives. Early case definitions for SARS-CoV-2 infection established by the World Health Organisation (WHO) included symptoms of fever and cough

requiring hospital admission and a recent travel history to Wuhan, Hubei Province, China.[201] The case definition for COVID-19 changed during the pandemic, incorporating newly developed laboratory tests and dropping exposure and symptom criteria as the outbreak expanded.

The operational gold standard for detecting SARS-CoV-2 infection soon became reverse-transcription polymerase chain reaction (RT-PCR) tests.[202] This laboratory technique converts SARS-CoV-2 viral RNA into cDNA by reverse transcription and amplifies pre-specified DNA targets using the polymerase chain reaction. At the start of the COVID-19 pandemic, different RT-PCR assays were used with varying sensitivity and specificity.[203] The usefulness of RT-PCR to diagnose SARS-CoV-2 infection was investigated almost exclusively in hospitalised and symptomatic patients. With the limited availability of RT-PCR tests in the first months of the pandemic, a symptom-based case definition was also used to detect SARS-CoV-2 infected cases in the UK during the first several months.[197,204]

In March 2020, lateral flow immunoassay devices (serology tests) were commercially developed for COVID-19. These tests could detect the presence of immunoglobulins (Ig) M and G neutralising antibodies against SARS-CoV-2. In one study, 76% of SARS-CoV-2 infected cases have IgM appear within 4-10 days of symptom onset and 100% within 11-15 days, with 87% persisting until 31-41 days.[205] IgG antibodies appear later and persist longer, with 65% of people having detectable levels within 4-10 days of symptom onset, up to 100% by 16-20 days and onwards. Several serology tests were developed in the early stages of the outbreak with highly variable performance.[206]

Before the COVID-19 pandemic, metagenomic next-generation sequencing (mNGS) technology had been proposed as a potential tool for detecting novel pathogens[67] This technique can also detect rare and co-infections not present on standard diagnostic panels. Unlike RT-PCR, which is limited to detecting known pathogens and requires a predetermined gene target, mNGS tools can sequence all the genetic material in a clinical sample. The mNGS approach can provide agnostic detection of new pathogens and remain robust in the presence of target gene mutation.[58] Techniques such as sequence-independent single-primer amplification (SISPA) randomly amplify small amounts of nucleic acids and improve mNGS sensitivity, although this tool is still not as sensitive as RT-PCR.[207] Nonetheless, mNGS was successfully used in multiple studies to diagnose patients presenting with unexplained pneumonia with SARS-CoV-2 infection early in 2020 in China.[59,60] Since then, mNGS tools have continued to improve with new methods such as BugSplit demonstrating the ability to detect SARS-CoV-2 in samples with high sensitivity and specificity even when reference sequences are unavailable.[61]

These four detection methods for SARS-CoV-2 infection (RT-PCR, the symptom-based case definition, serology tests and mNGS) are shown in Figure 4.1 and contrasted against the typical COVID-19 disease course. Each method offers a different detection window depending on when testing is conducted. For example, SARS-CoV-2 RNA can rise to detectable levels 2 - 3 days before symptom onset and peaks prior to the rise of antibodies.[208] This pre-symptomatic increase in viral load correlates with high infectiousness.[209] Serology positivity of IgG, and to a lesser extent IgM, can persist at detectable levels after cessation of symptoms and the clearance of the SARS-CoV-2 virus. Later deployed SARS-CoV-2 detection methods, such as rapid antigen lateral flow tests, are omitted as they only entered widespread use in the UK in December 2020.[210]

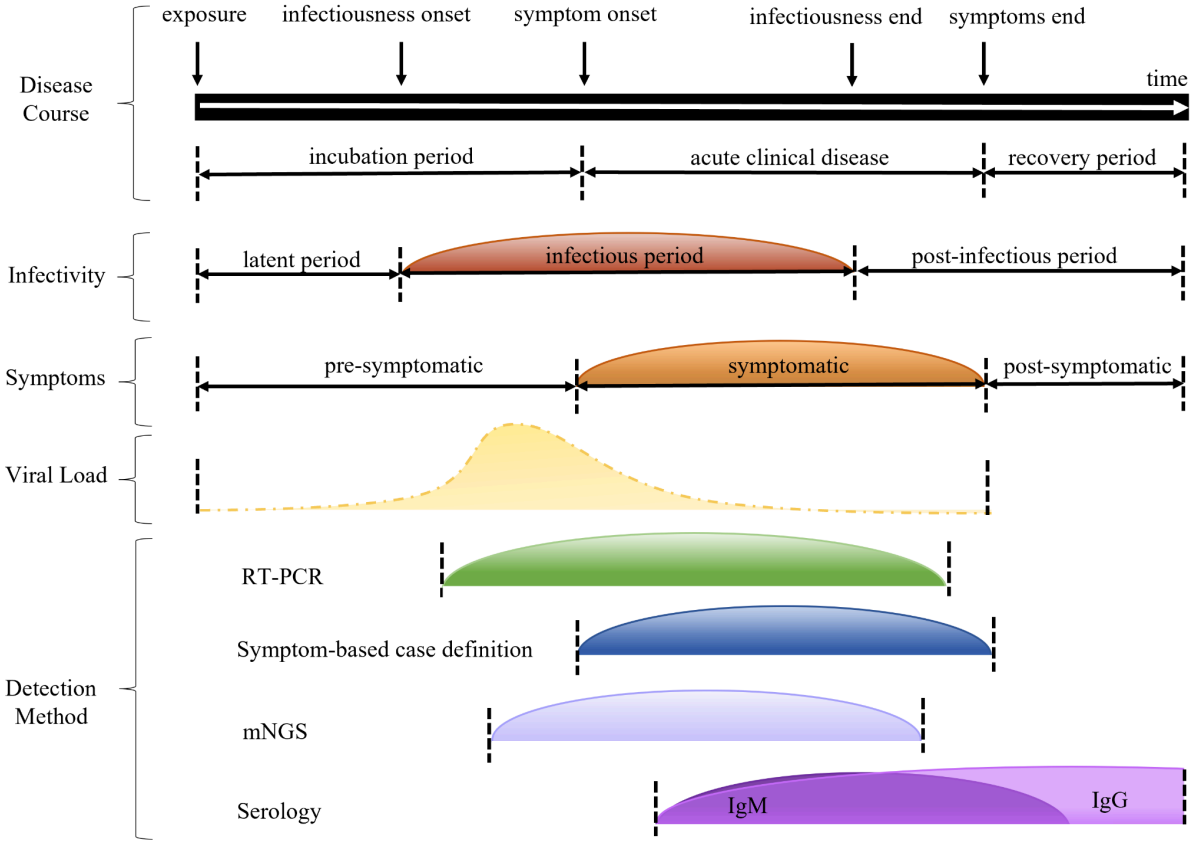


Figure 4.1 An illustrative diagram showing the main COVID-19 disease course milestones over time in a symptomatic case who recovers. Contrasted in colour are the infectious period (red), the symptomatic period (orange), the relative SARS-CoV-2 viral load (yellow) and the detection windows for RT-PCR (green), the symptom-based case definition (blue), mNGS (lavender) and serology with IgG (light purple) and IgM (dark purple) antibodies included. COVID-19: Coronavirus disease 2019; RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; mNGS: metagenomic next-generation sequencing; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Three of the four detection methods can be used in SARS-CoV-2 infection cases that remain completely asymptomatic throughout their disease course, the exception being the symptom-based case definition. In one study, asymptomatic infections were found to occur in 25% (95%CI: 16 – 38%) of cases after excluding pre-symptomatic tests.[211] However, estimates of asymptomatic infection vary significantly, from 4-80% of total SARS-CoV-2 infections.[212–215] In the early stages of a new pathogen outbreak, it is important to establish an asymptomatic case count, as these individuals may contribute to transmission in community settings. Asymptomatic COVID-19 cases are known to be infectious, although at lower levels than symptomatic cases.[216]

4.2.3 Microbiome Assessment

In addition to the possible detection of SARS-CoV-2 infection, mNGS tools also allow for the assessment of the background microbiome. Locations like the oropharynx host a community of different species, including pathogenic and non-pathogenic bacteria and viruses. The diversity of such communities can be estimated in various ways.[217] This paper highlighted three measures of species diversity: alpha (α) diversity being the similarity of species within a particular area; beta (β) diversity describing the change in community composition between different areas; and gamma (γ) diversity referring to the total species in the entire region. The oral microbiome has been shown to undergo detectable alpha and beta diversity alterations during SARS-CoV-2 infection.[218] The microbiome of the nasopharynx also undergoes significant beta diversity changes in the presence of SARS-CoV-2 and has detectable alterations based on disease severity.[219] However, this paper found no significant alpha diversity differences between hospital and community SARS-CoV-2 infected individuals nor their non-infected counterparts. Recent studies have called for further understanding of the oral microbial community changes in the presence of SARS-CoV-2 and investigation of potential microbiome-based diagnostic development.[220,221]

4.2.4 Health Behaviours

Behaviours which affect the chance of a person transmitting or becoming infected with a pathogen can change the prevalence of a disease like COVID-19. In the early stages of the pandemic, it was unclear which actions would most reduce the size of the outbreak. In early 2020 in the UK, public health advice shifted several times as the situation evolved and health behaviours were targeted at both the individual and population levels. Messaging to the public included instructions on hand hygiene, keeping a two-metre distance away from

others, socially isolating at home while unwell with COVID-19 symptoms, and later included mandatory social distancing and stay-at-home orders.[200] Community use of face masks was not initially recommended in UK public health advice.[222] When lockdown commenced in March 2020, there was an imperative to monitor compliance rates and establish predictors of adherence.[223–225] At the time, the potential effectiveness of lockdown was unknown, and it was unclear how compliance with various health behaviours would change over time.

4.2.5 Research Questions

Given the rapidly evolving situation in the UK, there was an imperative in March 2020 to answer various research questions about the COVID-19 pandemic. Given the unknowns regarding SARS-CoV-2 in the community at the time of study design, this research was conducted to answer the following:

1. What was the community prevalence of SARS-CoV-2 infection in April - June 2020 in a representative Oxfordshire, UK population?
2. How do alternative SARS-CoV-2 detection methods (serology, mNGS and the symptom-based case definition) compare with RT-PCR as the gold standard?
3. How does the oropharyngeal microbiome differ in those with evidence of SARS-CoV-2 infection as detected via different methods?
4. How do health behaviours change over the early stages of a pandemic?

Section 4.3 Methodology

This research was conducted as a serial cross-sectional observational study in the adult community population of Oxfordshire, UK. Sampling occurred over five time points (Rounds) at approximately fortnightly intervals between 20 April and 17 June 2020. Taking

place during the first national lockdown of the COVID-19 pandemic, the study design had to accommodate unprecedented disruptions to supply chains, courier availability, building and laboratory access and restrictions on human movement.

4.3.1 Sample Size Calculation

In March 2020, when this study was designed, the prevalence of SARS-CoV-2 infection in the UK community was unknown. A sample size calculation for prevalence was conducted using previously described methods.[226] With the first national lockdown not yet announced, the predicted lower bound for prevalence for 20 April 2020 onwards was assumed to be at least 0.04 (4%), given increasing case numbers in the UK with a doubling time of 3.8 days on 16 March 2020, and rapid progression in comparable European countries.[227,228] Because of the high uncertainty at the time of study design, a simple exponential model was constructed to estimate the future prevalence (Appendix D). With the first case on 23 January 2020 in the UK and a doubling time of 3.8 days, this predicted 11.39% prevalence by 20 April 2020. However, it was presumed that public health interventions would have some, albeit unknown, impact on reducing transmission before that level was reached, and study funding was secured in March 2020 assuming a more conservative 4% or higher prevalence level at the time of testing. The ONS COVID-19 Infection Survey later found community prevalence levels in England as high as 7.6% (95% CI 7.4 -7.8%) in March - April 2022, indicating this assumption at the time was not unreasonable.[229]

The sample size equation is shown in Equation 4.1, with n the sample size, Z the test statistic, P the estimated prevalence in the population, and d the precision required.

$$n = \frac{Z^2 P(1-P)}{d^2} \quad (4.1)$$

The sample size required for a 95% level of confidence (Z = 1.96) was calculated for a P of 0.04. The precision d was set to $\frac{P}{2}$ = 0.02 as recommended for when P is estimated to be <0.10.[226] This gives a minimum sample size required of 369 participants per Round.

Calculations were also conducted to determine the sample size required to evaluate different diagnostic test approaches using previously described methods.[230] RT-PCR tests were considered the gold standard and compared with three alternative diagnostic approaches: serology, mNGS and the symptom-based case definition. The sample size calculations for the sensitivity and specificity of these tests as compared against the gold standard are shown in Equation 4.2. Here, n is the sample size; S_n is the sensitivity; S_p is the specificity; Z *is* the test statistic; P *is* the estimated prevalence; and W *is* the clinically acceptable width of the CI for the sensitivity and specificity estimates.

$$n_{Sn} = \frac{Z^2 Sn(1-Sn)}{PW^2}$$

$$n_{Sp} = \frac{Z^2 Sp(1-Sp)}{(1-P)W^2}$$

$$n = max\{n_{Sn}, n_{Sp}\} \quad (4.2)$$

From Equation 4.2, for a given sample size, a sensitivity or specificity of 50% yields the maximum width of the CI. Because the sensitivity and specificity of the alternative diagnostic tests were unknown for SARS-CoV-2 infection in community populations, the conservative choice of 50% for both sensitivity and specificity was used as recommended.[230] For a P of

0.04 (4%) and W of 0.1 (10%) with 95% level of confidence ($Z = 1.96$), the minimum sample size required was 2401 participants total. Sampling this number would provide at least 96 true positive samples in expectation. Given community prevalence uncertainties at the start of the pandemic, the decision was made to sample up to 3000 participants to compare diagnostic approaches with a minimum of 400 participants per Round to determine community prevalence.

4.3.2 Participant Recruitment and Selection

From 1 April 2020, study participants were recruited through email circulation, radio announcements and dedicated Twitter and Facebook social media accounts. Prospective volunteers could visit a study-specific website hosted by the University of Oxford which provided research information, eligibility criteria and a link to a secure participant recruitment form through the Jisc online survey platform. The eligibility criteria for recruitment specified the need to be 18 years of age or older, a current UK resident, not currently admitted to a health facility and not having tested positive for COVID-19. For volunteers who confirmed they met these requirements, their age (in years), sex, and contact details were collected. A total of 10,711 applications were submitted.

Due to pandemic-related restrictions on human movement and courier coverage areas, the study was limited in April 2020 to residents of the Oxfordshire region. A total of 4,748 applicants were excluded for being residents outside Oxfordshire. A further 472 volunteers were excluded during data cleaning because of incorrect contact details, and 239 duplicate submissions were removed. Participant consent could be withdrawn at any stage prior to de-identification, which took place after the laboratory received samples; 79 volunteers withdrew from participation.

From the remaining 5,173 eligible recruits, 2,908 were selected to be sent test kits through stratified random sampling using the following method. Recruits were randomly assigned seven-digit unique participant identification numbers (IDs) and binned into sex and age groups 18-29, 30-44, 45-64 and 65+ years. A separate unique household linkage identifier was generated for volunteers from the same household address. Recruits were compared with 2011 census data, as shown in Table 4.1.[231] Based on the number of recruits in each stratum and courier accessibility constraints, the town of Oxford was sampled during the first four Rounds and the rest of Oxfordshire in the final fifth Round.

Table 4.1 Demographics of the Oxfordshire adult population showing age group and sex from the 2011 census with separate divisions for Oxford, the rest of Oxfordshire, and all total.[231]

Region & Age Group	Males	Females	Total
	n (%)	n (%)	N (%)
Oxford			
18 - 29 years	23,840 (19.3)	24,112 (19.5)	47,952 (38.8)
30 - 44 years	16,155 (13.1)	15,185 (12.3)	31,340 (25.4)
45 - 64 years	13,769 (11.1)	13,824 (11.2)	27,593 (22.3)
65+ years	7,224 (5.8)	9,416 (7.6)	16,640 (13.5)
Subtotal	*60,988 (49.4)*	*62,537 (50.6)*	*123,525 (100.0)*
Rest of Oxfordshire (Cherwell, South Oxfordshire, Vale of White Horse and West Oxfordshire)			
18 - 29 years	34,298 (8.7)	31,110 (7.9)	65,408 (16.7)
30 - 44 years	50,882 (13.0)	53,058 (13.5)	103,940 (26.5)
45 - 64 years	67,159 (17.1)	68,703 (17.5)	135,862 (34.6)
65+ years	39,412 (10.0)	47,690 (12.2)	87,102 (22.2)
Subtotal	*191,751 (48.9)*	*200,561 (51.1)*	*392,312 (100.0)*
Oxfordshire			
18 - 29 years	58,138 (11.3)	55,222 (10.7)	113,360 (22.0)
30 - 44 years	67,037 (13.0)	68,243 (13.2)	135,280 (26.2)
45 - 64 years	80,928 (15.7)	82,527 (16.0)	163,455 (31.7)
65+ years	46,636 (9.0)	57,106 (11.1)	103,742 (20.1)
Total	*252,739 (49.0)*	*263,098 (51.0)*	*515,837 (100.0)*

Sampling was limited by the number of addresses couriers could visit in a day (400), not the number of participants. To work within this constraint, 400 participant IDs were randomised to receive test kits using the R package *randomizeR*.[232] These participants were chosen proportional to the age and sex stratum from 2011 census data.[231] Other volunteers in the same household as those selected were then also included in the form of snowball sampling. Therefore, the sample size for each Round could vary based on the number of eligible volunteers in the same household. Sampling in postcode regions OX1, OX2, OX3 and OX4 took place on 20 April (Round 1) with 729 participants, 4 May (Round 2) with 552 participants, 18 May (Round 3) with 606 participants and 2 June 2020 (Round 4) with 483 participants. Testing in postcode regions OX5, OX10, OX11, OX12, OX13, OX14, OX26 and OX29 took place on 17 June 2020 (Round 5) with 538 participants.

4.3.3 Sampling Rounds

Recruited volunteers selected for the study were contacted via email the week before sampling. Each participant was informed of the testing date and procedure and reminded of the study requirements. Recruits who withdrew at this stage were replaced by a new randomised participant from the same sex and age category stratum to ensure adequate sampling.

Test kits were prepared for each participant. Each kit included a printed version of the research study overview, participant information sheet, consent form, and instructions on completing a serology test, collecting two oropharyngeal swabs, and how to package their samples for collection. Equipment placed in the kits included two individually packaged sterile flocked swabs, two specimen transport containers with 2ml of universal transport medium and guanidine thiocyanate (Prestige Diagnostics UK Ltd Cat No SSCCOV1)

pre-labelled with unique identifier barcodes, two disposable single-use finger lancets, an individually packaged serology test, buffer for the serology test, a plastic pipette, absorbent transport cushion, pre-labelled specimen bag, cardboard mail package with UN3373 label manufactured to biological transport specifications, addressed envelope for completed consent forms, tape and a pre-labelled mailbag.

Four serology test brands were used in this study based on availability. In Round 1, Orient Gene® Biotech COVID-19 IgG/IgM Rapid Test Cassettes and VivaDiag® COVID-19 IgM/IgG Rapid Test were used. For Rounds 2 and 3, Vazyme® 2019-nCoV IgG/IgM Detection Kits were used, while Rounds 4 and 5 used CTK Biotech OnSite® COVID-19 Ag Rapid Test. In Round 3, there was insufficient buffer available for the Vazyme® serology tests. The manufacturer was contacted, but supply chain issues meant more buffer could not be delivered; instead, instructions on preparation were obtained. More buffer was produced at a laboratory in Oxford according to the manufacturer's specifications for use in Round 3.

On each of the five sampling test days, couriers delivered packages to all participants who were asked to complete all study requirements that same day. Volunteers were asked to check over the contents of their kit, read the study information sheet and read and sign the consent form before proceeding. Instructions on completing swab collection and serology testing were provided in written form with pictures and links to video demonstrations created specifically for this study. Study investigator emails and phone numbers were provided to participants so that any queries about the instructions could be answered on the day of testing. After swab collection and serology testing, participants went online to complete the study questionnaire through a secure Jisc online survey platform.

The survey collected information on COVID-19 symptoms experienced in the preceding 14 days. Questions about risk factors, health behaviours, social distancing, and self-isolation were also asked. In addition, data on contact patterns with persons inside and outside the household was collected. Participants submitted information about their main economic activity, occupation, education level, smoking habits, chronic medical conditions, respiratory allergies and pregnancy. Information on household size, key worker status and contact with vulnerable populations was also collected. Participants were asked to report their serology results at the end of the survey after being provided pictures of potential valid and invalid outcomes, with an example for one brand, Vazyme®, shown in Figure 4.2. All survey questions were optional.

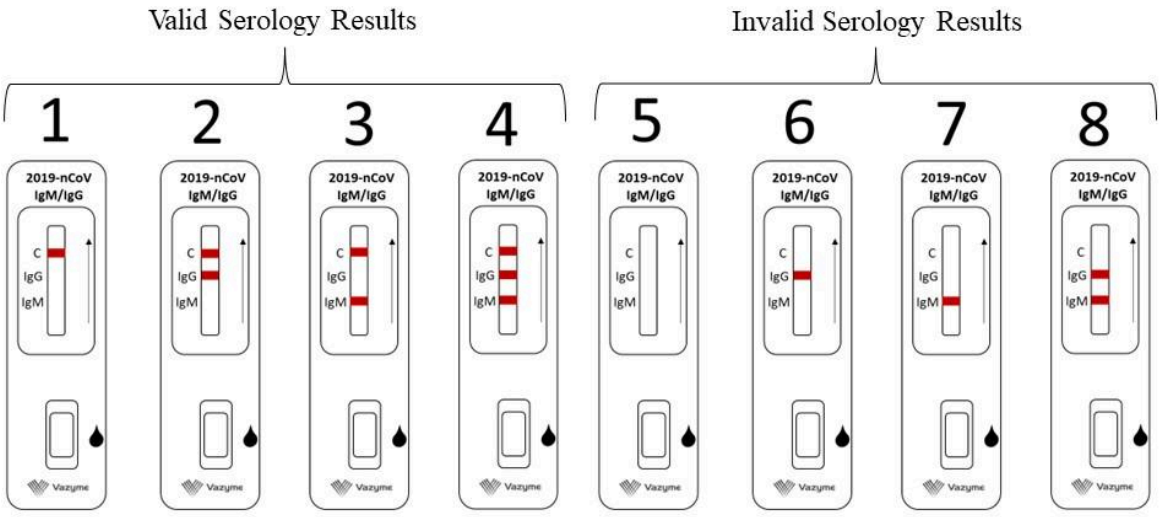


Figure 4.2 These images were provided to participants on the online survey when asked to report their serology test results after completing provided instructions. These images show valid (1 - 4) and invalid (5 - 8) results for Vazyme® 2019-nCoV IgG/IgM Detection Kits; participants supplied with other serology test brands were given images corresponding to their specific test. 2019-nCoV: 2019 novel coronavirus (Severe acute respiratory syndrome coronavirus 2); C: Control; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Participants followed instructions on safely packaging their two barcode-labelled specimen containers containing collected swabs according to UN3373 biological substance category B transport specifications with the materials provided to them. Containers were identical except for a designation of “P” or “N”, specifying RT-PCR or mNGS testing, respectively.

Completed consent forms were sealed separately in envelopes. Couriers returned to participant households to pick up all packages and envelopes the following day under non-contact procedures and returned them to the Peter Medawar Building for Pathogen Research. Samples were checked and processed in a Containment Level 3 laboratory following standard operating procedures and legislative, biosafety and regulatory protocols. Specimens were stored at -80 degrees Celsius, with their status and location tracked in an inventory catalogue.

4.3.4 Laboratory Tests

Swabs were transferred by international courier to the University of British Columbia (UBC) under cold-chain and UN3373 biological substance category B transport specifications. RT-PCR was carried out at the British Columbia Centre for Disease Control in collaboration with UBC. The protocol employed was based on amplified detection of the RNA-dependent RNA polymerase (*RdRp*) gene, envelope (*E*) gene and Ribonuclease P *(RNase P*), with C_T values produced for each. Both *RdRp* and *E* genes had to be detected for the result to be classified as positive. If only one of the *RdRP* or *E* genes were found, repeated testing runs were conducted until positive, negative or indeterminate results were reached.

A limited budget was available to carry out mNGS on a subset of participants. A total of 116 samples were chosen for sequencing using Oxford Nanopore Technologies MinION at UBC. All participants with RT-PCR positive or indeterminate results, IgM-only positive serology, or who met the symptom-based case definition for COVID-19 were sequenced. In addition, the household contacts of all PCR-positive participants underwent mNGS. The rationale for sequencing these participants was to investigate if there was any evidence of SARS-CoV-2

infection and to detect any notable changes in the microbiome. For comparison, matching with 4:1 controls for each RT-PCR positive case was carried out using the *ccoptimalmatch* R package using Round, age group, sex, main economic activity and education level.[233]

The Rapid Switching Mechanism at the 5′ end of RNA Template (SMART)-9N approach was used for mNGS sequencing as described previously.[234] The SMART-9N method allowed for the capture of total RNA in the sample, including from bacteria and RNA viruses. Up to twelve samples were multiplexed on each Nanopore flowcell. For each set of samples that underwent library preparation, one blank negative control that contained only water was included to control for reagent contamination during sample preparation. Basecalling of raw Oxford Nanopore Technologies (ONT) reads was performed using Guppy (Version 5.0.7, ONT) using the fast-basecalling setting. The basecalled nanopore reads from each sample were processed through BugSeq for metagenomic classification using the previously described algorithm for taxonomic assignment.[235] BugSeq performs crosstalk correction, adjusting for flowcell barcode crosstalk by controlling for the total number of dual-barcoded reads on each flowcell and assuming an even distribution of misclassification.[236] Human reads were removed prior to analysis by alignment against the human reference genome using *minimap2.*[237] After completion of BugSeq processing, metagenomic read-based classification tables summarising the taxonomic composition of each sample were extracted for downstream analysis.

4.3.5 Data Preparation

Raw data were explored and combined into appropriate groups for analysis. Household size was divided into 1, 2, 3, 4 or 5+ occupants. For main economic activity, full and part-time employment were grouped under “Paid employment.” Due to small cell counts, unemployed,

homemaker, long-term parental and sick leave were grouped with the "Other" category. For occupation categories, health and social care were grouped, as were business and administration. Education was grouped with professionals, which included lawyers, researchers and engineers. The remaining other specified jobs (hospitality, transportation, skilled labour, manual work and agriculture) were grouped with "Other" given low individual responses in each. Participants were asked about their key worker status after being provided with the UK government list describing key worker occupations.[238] The education category "Higher degree" grouped Masters, PhD and Medical Degrees.

All symptom data were examined and grouped as appropriate with the following COVID-19 symptoms included: fever, chills, runny nose, sore throat, cough, shortness of breath, headache, muscle/joint pain, feeling tired/malaise, coloured sputum/phlegm, gastrointestinal symptoms (loss of appetite, nausea, vomiting, diarrhoea) and loss of taste or smell. Only symptoms that were present on the day of the survey and had commenced within the last 14 days were included to limit to acute current presentations and exclude chronic disorders.

All health factors, including chronic medical conditions and risk factors, were examined separately and then grouped: chronic respiratory disease (chronic obstructive pulmonary disease, emphysema, asthma); cardiovascular disease (hypertension and coronary heart disease); other chronic disease (chronic neurological conditions, kidney or liver disease), diabetes; immunocompromised (HIV/AIDS, current chemotherapy treatment, sickle cell disease, splenectomy or steroid treatment); severe obesity with body mass index 40 or above, current pregnancy, current nicotine use (cigarette or e-cigarette); and chronic respiratory allergies (hay fever, dust mites, domestic animals or pets, or any other allergy that causes respiratory symptoms, sneezing, or watery eyes).

Participants were assessed to determine if they met the symptom-based case definition for SARS-CoV-2 infection. The criteria used were based on UK public health guidance at that time.[204] They included any of the following commencing within the previous 14 days and present on the day of the survey:

- a new continuous cough

  OR

- a fever with temperature ≥37.8°C

  OR

- a loss or change in taste (ageusia) or smell (anosmia)

Social distancing was defined in the study as a participant reporting in the preceding seven days: not leaving their property for reasons other than food, health, daily exercise or work; not visiting other households; and not having visitors to their home. This definition mirrored the lockdown rules in effect during most of the sampling period.[200] Social isolation was defined as: not leaving home for any reason because the participant or someone they live with has symptoms of COVID-19; and not having outside visitors to one's home. This definition mirrored the NHS self-isolation guidelines in effect during the study.[239]

Participants who had left their property in the preceding seven days were also asked about health behaviours. Two behaviours were analysed: frequency of washing hands on returning home from outside (designated "hand washing") and frequency of staying at least two metres away from other people who are not members of the participant's household (designated "two-metre distancing"). Participants could answer that they conducted this behaviour in the preceding seven days: Always (100% of the time), Mostly (about 75% of the time), Sometimes (about 50% of the time), Occasionally (about 25% of the time) or Never (0% of

the time) for each of these questions. Answers were examined separately and as a binary category of "Always" vs "Not Always", the latter combining the remaining four groups. For mNGS data, direct read counts assigned to specified taxon levels were used. Each sample was combined in R with taxonomy information obtained from the National Center for Biotechnology Information (NCBI) unique identifier number provided by Bugseq using the *taxonomizr* R package.[240] Because there is missing taxonomy information within the NCBI dataset, the taxallnomy algorithm was used to complete the taxonomic tree.[241] Final datasets were created for direct read counts and relative abundance of each Operational Taxonomic Unit (OTU) in the sequenced data. The relative abundance tables were further processed to produce data frames for the following taxon levels: species, genus, family, order, class, phylum and superkingdom.

4.3.6 Data Analysis

All data were analysed in RStudio with R Version 4.2.2.[122] Participant characteristics, health factors and COVID-19 symptoms were examined for the whole sample population and by Round. Evidence of SARS-CoV-2 infection was investigated using four detection methods with RT-PCR as the gold standard compared against three alternatives: serology, mNGS and the symptom-based case definition. For RT-PCR, C_T values ≤ 25 for the *RdRp* and *E* genes were considered "low" and indicative of a high viral load, with "high" C_T values ≥ 30 corresponding to a low viral load, and C_T values in between given an "intermediate" ranking consistent with previous research.[242] For mNGS, any sample with a nanopore read count > 0, where reads were taxonomically assigned to SARS-CoV-2, was considered positive. The equations used to compare RT-PCR to alternative detection methods are shown in Table 4.2.

Table 4.2 Table with equations used to compare alternative detection methods against RT-PCR as the gold standard. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; Sn: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Ac: Accuracy; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; DOR: Diagnostic odds ratio; FDR: False discovery rate.

		Gold Standard Test for SARS-CoV-2 (RT-PCR)		$FDR = \frac{FP}{TP + FP} = 1 - PPV$	
		Positive	Negative		
Alternative Detection Method	Positive	*True Positive (TP)*	*False Positive (FP)*	$PPV = \frac{TP}{TP + FP}$	$LR+ = \frac{Sn}{1 - Sp}$
	Negative	*False Negative (FN)*	*True Negative (TN)*	$NPV = \frac{TN}{FN + TN}$	$LR- = \frac{1-Sn}{Sp}$
		$Sn = \frac{TP}{TP + FN}$	$Sp = \frac{TN}{TN + FP}$	$Ac = \frac{TP + TN}{TP + FN + FP + TN}$	$DOR = \frac{LR+}{LR-}$

The diagnostic odds ratio (*DOR*) was computed because of its usefulness as a single metric to determine test performance independent of prevalence.[243] The DOR is the ratio of the odds of a positive result in participants with the disease relative to the odds of a positive result in the absence of disease. The DOR is based on the positive likelihood ratio (*LR+*) and negative likelihood ratio (*LR-*), which are used in clinical settings to assist in the interpretation of the post-test probability of disease given a result.[244] The LR+ is the probability of a diseased case testing positive divided by the probability of a non-diseased person testing positive. In contrast, the LR- is the probability of a diseased case testing negative divided by the probability of a non-diseased person testing negative.

The false discovery rate (*FDR*) was calculated in addition to the positive predictive value (*PPV*) to assist with result interpretation, given its showcased utility.[245,246] Through rearranging the equations in Table 4.2, for a given sensitivity (*Sn*) and specificity (*Sp*) of a

diagnostic test with a known prevalence (*Pr*) of a disease, *PPV*, negative predictive value (*NPV*) and Accuracy (*Ac*) were defined as shown in Equations 4.3, 4.4 and 4.5, respectively.

$$PPV = \frac{Sn*Pr}{Sn*Pr + (1-Sp)*(1-Pr)} \quad (4.3)$$

$$NPV = \frac{Sp*(1-Pr)}{Sp*(1-Pr) + Pr*(1-Sn)} \quad (4.4)$$

$$Ac = Sn * Pr + Sp * (1 - Pr) \quad (4.5)$$

Binomial confidence intervals (CIs) were generated using the Clopper-Pearson exact method where appropriate, including for the sensitivity, specificity and test accuracy calculations.[247] The log method was used to generate confidence intervals for all likelihood ratios, including the LR+, LR- and DOR using methods described previously.[243] For all alternative detection method predictive values, including the PPV and NPV, standard logit-transformed confidence intervals were generated.[248]

The four detection methods were combined to create a category of evidence of current or previous SARS-CoV-2 infection, designated "any evidence" and defined as having one or more of the following results: RT-PCR positive, positive serology (IgM, IgG or both), meeting the criteria for the symptom-based case definition or any SARS-CoV-2 reads detected via mNGS. The rationale behind this combined category is that, while all four methods could potentially detect current SARS-CoV-2 infection, the serology tests can also detect previous infections given persistent antibody production after viral clearance. This is especially true for IgG but can also occur for a shorter time with IgM antibodies.[205]

Multiple logistic regression was completed to compare participants with any evidence of current or previous infection vs no evidence as a dichotomous outcome variable. The null

hypothesis (H_0) was that for each *i* coefficient of β_i in the logistic regression model, H_0: $\beta_i = 0$, with the alternative hypothesis (H_A) being that H_A: $\beta_i \neq 0$. The reference group chosen for each variable was the normative or largest category, as appropriate. Generalised variation inflation factors (GVIFs) corrected by the number of coefficients degrees of freedom (*df*) were calculated for each predictor using the *car* R package.[249] All $GVIF^{1/(2df)} \geq 5$ were considered highly correlated and not included in the multiple logistic regression. For example, this included the independent variable "main economic activity" due to the multicollinearity of retired and student participants reporting their occupation as "none." All variables without high multicollinearity were included in the adjusted analysis irrespective of non-adjusted *p*-values obtained.

A *p*-value < 0.05 was considered significant for all statistical analyses.* For two-sample tests for equality of proportions, even when the event count frequency was low, Yates' Correction for Continuity was not used, given its longstanding history of poor statistical performance.[250,251] Fisher's exact test for count data was used when the expected frequency was < 5 in at least one cell in the contingency table being examined, as previously recommended.[252] For all generated 2x2 tables, *p*-values were calculated using Fisher's exact test. Larger contingency tables were analysed using the Freeman-Halton extension of Fisher's exact test.

* A *p*-value < 0.05 is the conventional threshold for determining statistical significance in many scientific disciplines, including public health. While this value is broadly recognised and ubiquitously used, it is potentially too liberal given it incurs an up to 5% chance of incorrectly rejecting the null hypothesis (Type I error) leading to false positive results. In addition, it can put too much emphasis on an artificially binary view of significant vs. non-significant rather than interpreting results within their full context. In this Chapter, to foster transparency and enable nuanced interpretation, all individual *p*-values ≥ 0.001 are reported throughout, allowing the reader to judge for themselves the degree of statistical evidence against the null hypothesis.

Participants from the same household were linked with a unique household ID. Due to the nature of snowball sampling and the heightened risk of infectious disease transmission within households, there was a concern that the collected data might not satisfy the typical statistical assumption of independence. To address potential clustering effects of SARS-CoV-2 infection within households, we employed a Generalised Estimating Equations (GEE) model for the seroprevalence analysis as this had sufficiently large counts. GEE models are particularly suitable for data where outcomes might be correlated due to clustering, as in cases where household members are likely to have correlated test outcomes because of linked exposure to a contagious disease. Unlike logistic mixed-effects models which focus on individual cluster effects, GEE provides a population-average of an effect while accounting for clustering. In this Chapter, the cluster analysis was performed using the *gee* package in R. Prevalence estimates, adjusted for household clustering, were obtained, and Wald 95% confidence intervals were derived using the standard error from the GEE model.

Microbiome comparisons were explored using four main comparison groups for mNGS participants: RT-PCR positive cases vs their matched negative controls; those who met the symptom-based case definition vs those who did not; participants with IgG-positive, IgM-positive, IgG and IgM-positive serology vs negative serology; and finally, those who had any evidence of current or previous SARS-CoV-2 infection vs those who did not. The H_0 for each was that there was no difference in microbiome between the groups.

Alpha diversity, the microbiome diversity within each sample, was examined in each group using four metrics: Richness, Shannon's diversity index, Evenness, and Simpson's diversity index. Richness is the count of the number of different species (or another taxon level) present in a sample. Shannon's diversity index is based on a 1948 formula for entropy and

provides an estimate of species diversity.[253] This is defined in Equation 4.6, where H is the Shannon diversity index; p_i is the proportion of individuals of i species in the sample; and S is the number of species, equivalent to Richness.

$$H = -\sum_{i=1}^{S} p_i ln\, p_i \tag{4.6}$$

From this equation, it can be seen that larger values of H indicate the sample has greater species diversity. Evenness (C) is a measure of the distribution of relative abundance across the species in a sample. C can be obtained by dividing the H by the natural logarithm of S. C is bounded [0,1], as seen in Equation 4.7.

$$C = \frac{-\sum_{i=1}^{S} p_i ln\, p_i}{ln\, S} \tag{4.7}$$

Species evenness will be high when all species (or other taxon level) in a sample have the same abundance, and approaches zero the more variance there is in relative abundance. The Simpson's diversity index takes into account the number of species and their relative abundance. In Equation 4.8, D is the Simpson diversity index with other variables defined above.

$$D = 1 - \sum_{i=1}^{S} p_i^2 \tag{4.8}$$

As the species richness and evenness increase, D will also increase. Note that the inverse of D is often computed, but for data analysis Equation 4.8 was chosen for use in the *vegan* R package.[254] Dissimilarity indices were computed using the Bray-Curtis metric to determine beta diversity. In Equation 4.9, the Bray-Curtis (BC) dissimilarity between two samples j and k is shown, with i the feature (such as OTUs), X_{ij} the frequency of i in j, and X_{ik} the frequency of i in k.

$$BC_{jk} = \frac{\sum_i |X_{ij} - X_{ik}|}{\sum_i (X_{ij} + X_{ik})} \quad (4.9)$$

After obtaining *BC* dissimilarity indices using the *vegan* package for different taxon levels, classical multidimensional scaling principal coordinate analysis (PCoA) was undertaken. This method takes *BC* dissimilarities imputed as a distance matrix and returns coordinates for visual representation in lower-dimensional Euclidean space. The amount of the variance explained by each axis is determined by the proportion of a given eigenvalue (λ_i) to the sum of all eigenvalues, as shown in Equation 4.10, where λ_i is the eigenvalue at index *i* within *n* total eigenvalues.

$$Variance\ explained = \frac{\lambda_i}{\sum_{i=1}^{n} \lambda_i} \quad (4.10)$$

For a two-dimensional PCoA plot, the x and y axes are derived from the top two eigenvalues with the variance explained by each axis derived using Equation 4.10. Visually, using this method, groups that are plotted closer to one another or show overlap have lower dissimilarity. The *vegan* package was used to conduct a permutational multivariate analysis of variance (PERMANOVA) at each taxon level to assess whether there was a significant difference between groups. This method takes computed distance matrices and assesses them using a model formula against variables of interest, in this case, the four comparison groups. A total of 9,999 permutations were used to compute the PERMANOVA *p*-values.

Differential abundance analysis (DAA) was undertaken to determine whether there were significant differences in abundance between groups of interest. First, centred log-ratio (CLR) transformed OTU data were obtained. The median standardised difference, designated the "effect size", was then calculated using the *ALDEx2* R package.[255] This measurement

takes the difference between two groups inputted as vectors and divides by a generated dispersion vector, outputting the median value as the effect size. This approach to effect size generation reduces the sensitivity of the result to differences in the underlying data distribution. The Kruskal-Wallis test was used to compare the four groups examined, generating an unadjusted *p*-value. This result was then compared against a Benjamini-Hochberg corrected *p*-value, designated *q*-value in the analysis, which takes into account the FDR controlling at significance level alpha (α) = 0.05. In this study, a *q*-value < 0.05 was deemed significant.

4.3.7 Ethics and Data Protection

Ethics approval for this study was received from the University of Oxford Medical Sciences Inter-Divisional Research Ethics Committee with reference R69035/RE007. The University of Oxford Central University Research Ethics Committee's best practice guidelines for research safety, internet-based research, data collection, protection and management and ethics review of research involving human tissue were used during the study. Contact details gathered through the online participant recruitment form were only used for arranging courier runs and kept secure in a password-protected file separate from other study data. All identifying details were deleted after participant samples were returned to the laboratory, leaving only de-identified participant barcode numbers. Samples were processed and stored at the Peter Medewar Building for Pathogen Research under Human Tissue Act Licence 12217. For RT-PCR and mNGS laboratory tests, ethical approval was received from the UBC Clinical Research Ethics Board with reference number H20-02152.

Section 4.4 Results

A total of 2,908 participants were included in this study. Of these, 1,559 (53.6%) were female, with ages ranging from 18 to 95 years (median 39 years, interquartile range [IQR]: 28 - 57 years). Of these 2,908 participants, a total of 2,678 (92.1%) completed the survey questionnaire; 2,606 (89.6%) reported serology test results; 2,713 (93.3%) returned swabs for RT-PCR; and 2,709 (93.2%) returned swabs for metagenomic analysis. Overall, a total of 1,746 (60.0%) volunteers came from a household where greater than one volunteer participated in the study, with a median of two and a maximum of six participants per household. The remaining 1,162 (40.0%) volunteers were the only participating member of their household.

4.4.1 Participant Characteristics

The participant characteristics of the entire sample population are summarised in Table 4.3. Among those who completed the survey (n = 2,678), the median household size was two occupants. The majority (58.6%) of participants stated their main economic activity as paid employment, and 18.3% reported being key workers. After limiting to participants in paid employment, 429/1569 (27.3%) stated they were key workers. These results are comparable to 2011 UK census data which showed 56.0% of adults in Oxfordshire in paid employment and 28.9% being key workers.[238,256] The most commonly reported occupation was professional (37.5%), followed by “none” (32.4%), which included full-time students, retired persons as well as unemployed individuals. The majority (54.3%) of study participants had obtained a higher degree. For comparison, 2011 census data showed 42.6% of Oxford adults with this education level.[257]

Table 4.3 Participant characteristics of the sample population. Sex and age group data were available for all participants, with the remaining information limited to those who completed the survey, as indicated in brackets.

Characteristics	Sample Population	
	Number	Percentage
Sex (*N* = 2908)		
Female	1559	53.6%
Male	1349	46.4%
Age Group, years (*N* = 2908)		
18 - 29	915	31.5%
30 - 44	795	27.3%
45 - 64	757	26.0%
65+	441	15.2%
Completed Survey (*N* = 2908)		
Yes	2678	92.1%
No	230	7.9%
Household Size, number of occupants (*n* = 2678)		
1	186	6.9%
2	1042	38.9%
3	499	18.6%
4	546	20.4%
5+	405	15.1%
Key Worker (*n* = 2678)		
Yes	491	18.3%
No	2142	80.0%
Unknown	45	1.7%
Main Economic Activity (*n* = 2678)		
Paid employment	1569	58.6%
Self-employed	245	9.1%
Student	318	11.9%
Retired	395	14.7%
Other	151	5.6%
Occupation (*n* = 2678)		
Health or social care	233	8.7%
Professional	1004	37.5%
Business or administration	330	12.3%
Other	244	9.1%
None	867	32.4%
Highest level of education obtained (*n* = 2678)		
O-levels or equivalent	105	3.9%
A-levels or equivalent	278	10.4%
Bachelor's degree or equivalent	814	30.4%
Higher degree	1454	54.3%
None of the above	27	1.0%

The participant characteristics for each Round is presented in Table 4.4. Survey completion decreased from 97.7% in Round 1 over each time point to 82.3% by Round 5. The majority of participants in each Round were female (51.4 - 57.1%), working in paid employment (53.1 - 61.9%) and not a key worker (77.0 - 84.4%). The most frequently reported household size in each Round was two occupants (36.8 - 41.3%), and the most common occupation was professional (35.3 - 40.2%). Round 5, conducted in Oxfordshire outside of Oxford, was similar to previous Rounds 1 - 4 by many measures but differed by having more participants reporting being retired (17.6% vs 13.0 - 14.7%) and fewer students (3.8% vs 9.9 - 16.3%). In addition, when comparing Round 5 vs Rounds 1 - 4, fewer participants (39.7% vs 56.3 - 59.1%) reported having a higher degree, while more stated their highest educational attainment to be O-levels or equivalent (9.3% vs 2.1 - 4.0%).

Table 4.4 Participant characteristics of the Oxfordshire study population by testing Round. Sex and age group data were available for all participants, with the remaining information limited to those who completed the survey, as indicated in **bold**.

Characteristics	Round 1	Round 2	Round 3	Round 4	Round 5*
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Total participants	**n = 729**	**n = 552**	**n = 606**	**n = 483**	**n = 538**
Sex					
Female	380 (52.1)	284 (51.4)	336 (55.4)	276 (57.1)	283 (52.6)
Male	349 (47.9)	268 (48.6)	270 (44.6)	207 (42.9)	255 (47.4)
Age Group, years					
18 - 29	264 (36.2)	203 (36.8)	192 (31.7)	140 (29.0)	116 (21.6)
30 - 44	186 (25.5)	139 (25.2)	152 (25.1)	141 (29.2)	177 (32.9)
45 - 64	176 (24.1)	131 (23.7)	167 (27.6)	123 (25.5)	160 (29.7)
65+	103 (14.1)	79 (14.3)	95 (15.7)	79 (16.4)	85 (15.8)
Completed Survey					
Yes	712 (97.7)	530 (96.0)	565 (93.2)	428 (88.6)	443 (82.3)
No	17 (2.3)	22 (4.0)	41 (6.8)	55 (11.4)	95 (17.7)
Responding participants	**n = 712**	**n = 530**	**n = 565**	**n = 428**	**n = 443**
Household Size, number of occupants					
1	19 (2.7)	39 (7.4)	42 (7.4)	53 (12.4)	33 (7.4)
2	280 (39.3)	219 (41.3)	217 (38.4)	163 (38.1)	163 (36.8)
3	137 (19.2)	88 (16.6)	115 (20.4)	75 (17.5)	84 (19.0)
4	141 (19.8)	109 (20.6)	112 (19.8)	81 (18.9)	103 (23.3)
5+	135 (19.0)	75 (14.2)	79 (14.0)	56 (13.1)	60 (13.5)
Key Worker					
Yes	100 (14.0)	103 (19.4)	105 (18.6)	87 (20.3)	96 (21.7)
No	601 (84.4)	419 (79.1)	448 (79.3)	333 (77.8)	341 (77.0)
Unknown	11 (1.5)	8 (1.5)	12 (2.1)	8 (1.9)	6 (1.4)
Main Economic Activity					
Paid employment	378 (53.1)	303 (57.2)	350 (61.9)	265 (61.9)	273 (61.6)
Self-employed	77 (10.8)	40 (7.5)	50 (8.8)	30 (7.0)	48 (10.8)
Student	116 (16.3)	82 (15.5)	56 (9.9)	47 (11.0)	17 (3.8)
Retired	105 (14.7)	69 (13.0)	80 (14.2)	63 (14.7)	78 (17.6)
Other	36 (5.1)	36 (6.8)	29 (5.1)	23 (5.4)	27 (6.1)
Occupation					
Health or social care	53 (7.4)	52 (9.8)	55 (9.7)	40 (9.3)	33 (7.4)
Professional	251 (35.3)	202 (38.1)	227 (40.2)	160 (37.4)	164 (37.0)
Business or administration	86 (12.1)	46 (8.7)	62 (11.0)	62 (14.5)	74 (16.7)
Other	65 (9.1)	43 (8.1)	54 (9.6)	32 (7.5)	50 (11.3)
None	257 (36.1)	187 (35.3)	167 (29.6)	134 (31.3)	122 (27.5)
Highest level of education obtained					
O-levels or equivalent	20 (2.8)	15 (2.8)	12 (2.1)	17 (4.0)	41 (9.3)
A-levels or equivalent	68 (9.6)	55 (10.4)	38 (6.7)	38 (8.9)	79 (17.8)
Bachelor's degree or equivalent	218 (30.6)	142 (26.8)	188 (33.3)	125 (29.2)	141 (31.8)
Higher degree	401 (56.3)	313 (59.1)	321 (56.8)	243 (56.8)	176 (39.7)
None of the above	5 (0.7)	5 (0.9)	6 (1.1)	5 (1.2)	6 (1.4)

*Round 5 was conducted in Oxfordshire in postcode regions OX5, OX10, OX11, OX12, OX13, OX14, OX26 and OX29 compared with Rounds 1 - 4 conducted in Oxford postcode regions OX1, OX2, OX3 and OX4.

Participants who completed the survey reported on health factors, as summarised in Table 4.5. Individuals with a chronic respiratory condition varied between 9.1 - 13.5% by Round. Overall, 20.6 - 26.9% of participants reported having one or more of the listed chronic medical conditions. When asked about nicotine consumption, 5.4 - 9.6% of participants reported current cigarette or e-cigarette use. In total, 44.2 - 49.6% of participants had at least one chronic respiratory allergy, the most common being hay fever. A small number of female participants (0.3 - 1.8%) in each Round reported being pregnant.

Table 4.5 Reported participant health factors by testing Round. The percentages are based on the number of responding participants in each Round, as shown in **bold**. Variables in *italics* indicate the presence of one or more health factors in the corresponding category.

Health Factor	Round 1	Round 2	Round 3	Round 4	Round 5
	n (%)	n (%)	n (%)	n (%)	n (%)
Responding Participants	**n = 712**	**n = 530**	**n = 565**	**n = 428**	**n = 443**
Chronic medical condition					
Chronic respiratory condition*	96 (13.5)	66 (12.5)	64 (11.3)	39 (9.1)	47 (10.6)
Cardiovascular disease	59 (8.3)	29 (5.5)	47 (8.3)	31 (7.2)	54 (12.2)
Other chronic disease†	11 (1.5)	7 (1.3)	7 (1.2)	2 (0.47)	4 (0.9)
Diabetes	12 (1.7)	9 (1.7)	6 (1.1)	9 (2.1)	14 (3.2)
Immunocompromised‡	6 (0.8)	14 (2.6)	10 (1.8)	6 (1.4)	5 (1.1)
Severe obesity	8 (1.1)	6 (1.1)	6 (1.1)	10 (2.3)	15 (3.4)
Any chronic medical condition	*166 (23.3)*	*116 (21.9)*	*120 (21.2)*	*88 (20.6)*	*119 (26.9)*
Current nicotine use					
Current smoker (cigarettes)	42 (5.9)	41 (7.8)	31 (5.5)	16 (3.7)	24 (5.4)
Current vaper (e-cigarettes)	27 (3.8)	16 (3.0)	14 (2.5)	8 (1.9)	14 (3.2)
Any current cigarette or e-cigarette use	*64 (9.0)*	*51 (9.6)*	*44 (7.8)*	*23 (5.4)*	*35 (7.9)*
Chronic respiratory allergies					
Hay fever	281 (39.5)	222 (41.9)	228 (40.4)	188 (43.9)	174 (39.3)
Other respiratory allergy§	169 (23.7)	127 (24.0)	125 (22.1)	85 (19.9)	76 (17.2)
Any chronic respiratory allergies	*341 (47.9)*	*263 (49.6)*	*264 (46.7)*	*204 (47.7)*	*196 (44.2)*
Responding Female Participants	**n = 358**	**n = 263**	**n = 309**	**n = 244**	**n = 227**
Pregnant	2 (0.6)	2 (0.8)	1 (0.3)	1 (0.4)	4 (1.8)

*Includes asthma, chronic obstructive pulmonary disease, emphysema or chronic bronchitis
†Includes chronic kidney disease, chronic liver disease or a chronic neurological condition
‡Includes HIV/AIDS, current chemotherapy treatment, sickle cell disease, splenectomy or steroid treatment
§Includes any other chronic allergy which causes respiratory symptoms including those caused by dust mites, domestic animals or other environmental factors

Participants who completed the survey reported whether they had experienced any acute COVID-19 symptoms in the preceding fourteen days. Table 4.6 summarises symptoms which were still current on the day of the survey by Round. The most commonly reported symptom was rhinorrhoea (runny nose), followed by tiredness/malaise and headache. The least common symptom was fever. In total, 11.3 - 20.1% of participants reported one or more current acute symptoms in each Round.

Table 4.6 Reported current acute COVID-19 symptoms from responding participants commencing within the preceding 14 days and present on the survey day. **Bold** indicates the number of responding participants, while *italics* indicate the presence of one or more of the listed symptoms. COVID-19: Coronavirus disease 2019.

Symptom	Round 1	Round 2	Round 3	Round 4	Round 5
	n (%)	n (%)	n (%)	n (%)	n (%)
Responding Participants	**n = 712**	**n = 530**	**n = 565**	**n = 428**	**n = 443**
Fever	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)
Chills	4 (0.6)	1 (0.2)	1 (0.2)	1 (0.3)	1 (0.2)
Runny nose	67 (9.4)	38 (7.2)	29 (5.1)	42 (9.8)	17 (3.8)
Sore throat	30 (4.2)	18 (3.4)	18 (3.2)	16 (3.7)	3 (0.7)
Cough	28 (3.9)	14 (2.6)	14 (2.5)	11 (2.6)	2 (0.5)
Shortness of breath	9 (1.3)	4 (0.8)	4 (0.7)	7 (1.6)	4 (0.9)
Headache	44 (6.2)	18 (3.4)	20 (3.5)	15 (3.5)	13 (2.9)
Muscle/joint pain	26 (3.7)	14 (2.6)	21 (3.7)	11 (2.6)	10 (2.3)
Tiredness/malaise	31 (4.4)	24 (4.5)	21 (3.7)	23 (5.4)	12 (2.7)
Gastrointestinal symptom(s)*	15 (2.1)	4 (0.8)	11 (1.9)	8 (1.9)	7 (1.6)
Sputum/phlegm production	4 (0.6)	5 (0.9)	3 (0.5)	1 (0.2)	1 (0.2)
Loss of taste or smell	1 (0.1)	2 (0.4)	4 (0.7)	1 (0.2)	1 (0.2)
Any current acute symptom	*140 (19.7)*	*93 (17.5)*	*90 (15.9)*	*86 (20.1)*	*50 (11.3)*

* Includes nausea, vomiting, diarrhoea or loss of appetite

4.4.2 Evidence of SARS-CoV-2 Infection

Study participants were investigated for evidence of current or previous SARS-CoV-2 infection using four detection methods: RT-PCR, serology, mNGS and the symptom-based case definition. The results obtained using each detection method by Round are summarised

in Table 4.7 and explored separately in subsequent sections. Overall, any evidence of current or previous SARS-CoV-2 infection ranged from 10.1% in Round 1 to 4.7% in Round 5.

Table 4.7 Evidence of current or previous SARS-CoV-2 infection using four different detection methods. **Bold** indicates the number of participants, while variables in *italics* indicate the presence of any one or more of the preceding items in the corresponding category. RT-PCR: reverse-transcription polymerase chain reaction; IgG: Immunoglobulin G; IgM: Immunoglobulin M; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Detection Method	Round 1	Round 2	Round 3	Round 4	Round 5
	n (%)	n (%)	n (%)	n (%)	n (%)
Total participants	**n = 729**	**n = 552**	**n = 606**	**n = 483**	**n = 538**
RT-PCR					
Positive	4 (0.5)	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Negative	698 (95.7)	523 (94.7)	572 (94.4)	430 (89.0)	455 (84.6)
Invalid result	8 (1.1)	6 (1.1)	3 (0.5)	8 (1.6)	4 (0.7)
No result	19 (2.6)	21 (3.8)	31 (5.1)	45 (9.3)	79 (14.7)
Serology					
IgM positive only	7 (1.0)	10 (1.8)	7 (1.2)	5 (1.0)	2 (0.4)
IgM and IgG positive	26 (3.6)	6 (1.1)	0 (0.0)	5 (1.0)	2 (0.4)
IgG positive only	11 (1.5)	20 (3.6)	8 (1.3)	23 (4.8)	14 (2.6)
Positive (any serology)	*44 (6.0)*	*36 (6.5)*	*15 (2.5)*	*33 (6.8)*	*18 (3.3)*
Negative	620 (85.0)	461 (83.5)	135 (22.3)	382 (79.1)	396 (73.6)
Invalid result	15 (2.1)	31 (5.6)	408 (67.3)[†]	9 (1.9)	3 (0.5)
No result	50 (6.9)	24 (4.3)	48 (7.9)	59 (12.2)	121 (22.5)
Symptom-based case definition					
New cough	28 (3.8)	14 (2.5)	14 (2.3)	11 (2.3)	2 (0.4)
Loss of taste or smell	1 (0.1)	2 (0.4)	4 (0.7)	1 (0.2)	1 (0.2)
Fever (≥37.8°C)	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)
Positive (meets any symptom criteria)	*29 (4.0)*	*15 (2.7)*	*15 (2.5)*	*11 (2.3)*	*4 (0.7)*
Negative	683 (93.7)	515 (93.3)	550 (90.8)	417 (86.3)	439 (81.6)
No result	17 (2.3)	22 (4.0)	41 (6.8)	55 (11.4)	95 (17.7)
Metagenomic next-generation sequencing					
Positive	3 (0.4)	2 (0.4)	2 (0.3)	1 (0.2)	0 (0.0)
Negative	48 (6.6)	32 (5.8)	8 (1.3)	14 (2.9)	6 (1.1)
No result	678 (93.0)	518 (93.8)	596 (98.3)	468 (96.9)	532 (98.9)
All detection methods combined*					
Any evidence of SARS-CoV-2 infection	73 (10.0)	50 (9.1)	31 (5.1)	43 (8.9)	22 (4.1)
No evidence	647 (88.8)	490 (88.8)	554 (91.4)	405 (83.9)	451 (83.8)
No result	9 (1.2)	12 (2.2)	21 (3.5)	35 (7.2)	65 (12.1)

* Any evidence of current or previous SARS-CoV-2 infection includes all participants who were RT-PCR positive, had any positive serology (IgM, IgG or both), met the symptom-based case definition or had any SARS-CoV-2 reads detected via metagenomic next-generation sequencing

[†] Includes 379 participants provided laboratory-created buffer

As discussed in the Methodology, supply chain issues limited the amount of serology assay buffer available for Round 3. After conducting the study, the serology results obtained in Round 3 were investigated to determine whether the laboratory-created buffer (LCB) differed from the manufacturer-supplied buffer (MSB), with H_0 being no difference. Serology results were reported by 529/606 (87.3%) participants in this Round, with 150 (28.4%) receiving MSB and the remaining 379 (71.6%) receiving LCB through random allocation. From those who received the MSB, 15 (10.0%) were positive for any serology compared with 75 (19.8%) in those receiving LCB. A two-tailed test for equality of proportions for serology positive without Yates's correction for continuity gave a *p*-value of 0.0069 and 95% CI of (-0.16 to -0.04), indicating H_0 should be rejected and that there was a significant difference between manufacturer-supplied and laboratory-created buffer. LCB results for Round 3 were therefore counted as invalid in the data analysis.

Logistic regressions for individual detection methods, such as RT-PCR positivity, were not attempted because of the rarity of the events leading to low cell counts. Instead, univariate logistic regression was completed for predictors of any evidence of current or previous infection vs no evidence, as seen in Tables 4.8 and 4.9. Participants who received LCB were not included in this analysis. Variables with high multicollinearity were excluded from the multiple logistic regression analysis, as detailed in the methodology.

Table 4.8 Multiple logistic regression analysis on predictors for any evidence of current or previous infection vs no evidence. Any evidence of current or previous SARS-CoV-2 infection includes all participants who were RT-PCR positive, had any positive serology (IgM, IgG or both), met the symptom-based case definition or had any SARS-CoV-2 reads detected via metagenomic next-generation sequencing and was treated as a dichotomous variable in the analysis. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IgG: Immunoglobulin G; IgM: Immunoglobulin M; OR: Odds Ratio; CI: Confidence Interval.

Variable	Unadjusted Analysis			Adjusted Analysis*		
	β Coefficient	Unadjusted OR (95% CI)	*p*-value	β Coefficient	Adjusted OR (95% CI)	*p*-value
Sex						
Female	Referent	1	-	-	-	-
Male	-0.087	0.92 (0.69 - 1.2)	0.55	-0.094	0.91 (0.67 - 1.2)	0.55
Age						
Years	-0.0044	1.0 (0.99 - 1.0)	0.31	-0.0048	1.0 (0.98 - 1.0)	0.36
Round						
1	Referent	1	-	-	-	-
2	-0.10	0.9 (0.62 - 1.3)	0.60	-0.069	0.93 (0.62 - 1.4)	0.74
3	0.0097	1 (0.57 - 1.7)	0.97	0.12	1.1 (0.62 - 2.0)	0.68
4	-0.061	0.94 (0.63 - 1.4)	0.76	-0.055	0.95 (0.6 - 1.5)	0.81
5	-0.84	0.43 (0.26 - 0.7)	0.00083	-0.78	0.46 (0.26 - 0.79)	0.0062
Household Size (number of occupants)						
1	Referent	1	-	-	-	-
2	-0.39	0.68 (0.4 - 1.2)	0.16	-0.32	0.73 (0.41 - 1.4)	0.29
3	-0.52	0.59 (0.32 - 1.1)	0.098	-0.49	0.61 (0.32 - 1.2)	0.15
4	-0.30	0.74 (0.42 - 1.4)	0.32	-0.23	0.80 (0.43 - 1.5)	0.49
5+	0.11	1.1 (0.63 - 2.0)	0.72	0.013	1.0 (0.54 - 2.0)	0.97
Key Worker						
No or Unknown	Referent	1	-	-	-	-
Yes	-0.0049	1.0 (0.68 - 1.4)	0.98	0.23	1.3 (0.78 - 2.0)	0.33
Main Economic Activity						
Paid Employment	Referent	1	-	-	-	-
Self-employed	-0.33	0.72 (0.39 - 1.2)	0.26	-	-	-
Student	0.11	1.1 (0.72 - 1.7)	0.62	-	-	-
Retired	-0.064	0.94 (0.6 - 1.4)	0.77	-	-	-
Other	0.27	1.3 (0.71 - 2.2)	0.36	-	-	-
Occupation						
Professional	Referent	1	-	-	-	-
Health or social care	-0.47	0.62 (0.33 - 1.1)	0.13	-0.59	0.55 (0.26 - 1.1)	0.10
Business/administration	-0.62	0.54 (0.30 - 0.90)	0.023	-0.58	0.56 (0.30 - 0.98)	0.053
Other	-0.41	0.66 (0.36 - 1.1)	0.16	-0.26	0.77 (0.40 - 1.4)	0.41
None	-0.10	0.9 (0.65 - 1.2)	0.54	-0.0053	1.0 (0.68 - 1.4)	0.98
Highest level of education obtained						
Higher degree	Referent	1	-	-	-	-
Bachelor's or equivalent	-0.033	0.97 (0.70 - 1.3)	0.84	-0.0070	0.99 (0.69 - 1.4)	0.97
A-levels or equivalent	-0.20	0.82 (0.48 - 1.3)	0.42	-0.30	0.74 (0.39 - 1.3)	0.33
O-levels or equivalent	-0.15	0.86 (0.38 - 1.7)	0.69	0.34	1.4 (0.59 - 3.0)	0.40
None	-0.78	0.46 (0.025 - 2.2)	0.45	-0.49	0.62 (0.033 - 3.2)	0.64

* Adjusted for sex, age, Round, household size, key worker status, occupation, education level, chronic medical conditions, current nicotine use, chronic respiratory allergies, social distancing, social isolation, handwashing and two-metre distancing.

Table 4.9 Multiple logistic regression analysis on predictors for any evidence of current or previous infection vs no evidence. Any evidence of current or previous SARS-CoV-2 infection includes all participants who were RT-PCR positive, had any positive serology (IgM, IgG or both), met the symptom-based case definition or had any SARS-CoV-2 reads detected via metagenomic next-generation sequencing and was treated as a dichotomous variable in the analysis. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IgG: Immunoglobulin G; IgM: Immunoglobulin M; OR: Odds Ratio; CI: Confidence Interval.

Variable	Unadjusted Analysis			Adjusted Analysis*		
	β Coefficient	Unadjusted OR (95% CI)	*p*-value	β Coefficient	Adjusted OR (95% CI)	*p*-value
Chronic medical condition						
No	Referent	1	-	-	-	-
Yes	-0.15	0.86 (0.60 - 1.2)	0.39	-0.090	0.91 (0.61 - 1.3)	0.65
Current nicotine use						
No	Referent	1	-	-	-	-
Yes	0.027	1.0 (0.59 - 1.7)	0.92	-0.012	0.99 (0.53 - 1.7)	0.97
Chronic respiratory allergies						
No	Referent	1	-	-	-	-
Yes	0.22	1.2 (0.94 - 1.7)	0.13	0.23	1.3 (0.93 - 1.7)	0.14
Serology brand						
VivaDiag®	Referent	1	-	-	-	-
OnSite®	-0.70	0.50 (0.33 - 0.77)	0.0013	-	-	-
Orient Gene®	-0.54	0.58 (0.36 - 0.95)	0.030	-	-	-
Vazyme®	-0.40	0.67 (0.44 - 1.0)	0.063	-	-	-
Social distancing						
No	Referent	1	-	-	-	-
Yes	0.077	1.1 (0.80 - 1.5)	0.62	-0.16	0.86 (0.60 - 1.2)	0.38
Social isolation						
No	Referent	1	-	-	-	-
Yes	0.61	1.8 (1.1 - 3.0)	0.023	0.50	1.6 (0.76 - 3.2)	0.17
Hand washing; Frequency - Always						
No	Referent	1	-	-	-	-
Yes	-0.13	0.88 (0.64 - 1.2)	0.43	-0.24	0.79 (0.56 - 1.1)	0.17
Two-metre distancing; Frequency - Always						
No	Referent	1	-	-	-	-
Yes	0.3	1.4 (1.0 - 1.8)	0.051	0.3	1.4 (0.98 - 1.9)	0.068

* Adjusted for sex, age, Round, household size, key worker status, occupation, education level, chronic medical conditions, current nicotine use, chronic respiratory allergies, social distancing, social isolation, handwashing and two-metre distancing.

As can be seen in Table 4.8, participants who worked in business or administration were almost half as likely to have evidence of current or previous SARS-CoV-2 infection when compared with professionals, with an unadjusted OR (95%CI) of 0.54 (0.3 - 0.9) and *p*-value of 0.023. However, this relationship attenuated in the adjusted analysis with a *p*-value of 0.053. Round 5 participants were significantly less likely to have any evidence of current or

previous SARS-CoV-2 infection with an adjusted OR (95%CI) of 0.46 (0.26 - 0.79) and *p*-value of 0.0062. This may be due to the rural location for Round 5 compared with Rounds 1 - 4. Other results from this regression are discussed in subsequent relevant sections.

4.4.2.1 Gold Standard Detection using RT-PCR

From the 2,713 participants who underwent SARS-CoV-2 RT-PCR testing, 2,678 (98.7%) were negative, and 29 (1.1%) had invalid results (3 indeterminate and 26 poor quality). Six participants (0.22%) returned a positive RT-PCR result. These six samples had C_T values ranging from 25.7 - 37.8 (mean 32.7) for the *RdRp* gene, 25.4 - 38.7 (mean 32.5) for the *E* gene and 27.6 - 31.6 (mean 29.2) for the *RNase P* gene. Overall, 2/6 (33.3%) of RT-PCR positive individuals had intermediate C_T values for the *RdRp* and *E* genes, while 4/6 (66.7%) had high C_T values ≥ 30, indicating intermediate and low viral loads, respectively. None of the participants had low C_T values which would have indicated high viral loads.

After limiting to participants with valid RT-PCR results (excluding those with invalid or no result), those testing positive with 95%CI calculated using the exact binomial test was 4/702 (0.57%, 95%CI [0.16 - 1.45%]) positive on 20 April 2020, 2/525 (0.38%, 95%CI [0.046 - 1.37%]) positive on 4 May 2020, 0/572 (0.0%, 95%CI [0.0 - 0.064%]) on 18 May 2020, 0/430 (0.0%, 95%CI [0.0 - 0.85%]) on 2 June 2020 and 0/455 (0.0%, 95%CI [0.0 - 0.81%]) on 17 June 2020. This serial prevalence data were compared in Figure 4.3 with the ONS COVID-19 Infection Survey daily modelled data for the whole of England and the South East region, which commenced on 26 April 2020.[258] Oxford and Oxfordshire are contained within the South East region of England, and the ONS had no lower granularity data available for this period. As can be seen, the SARS-CoV-2 infection prevalence estimates obtained in this study are consistent with the ONS results for England and the South East region.

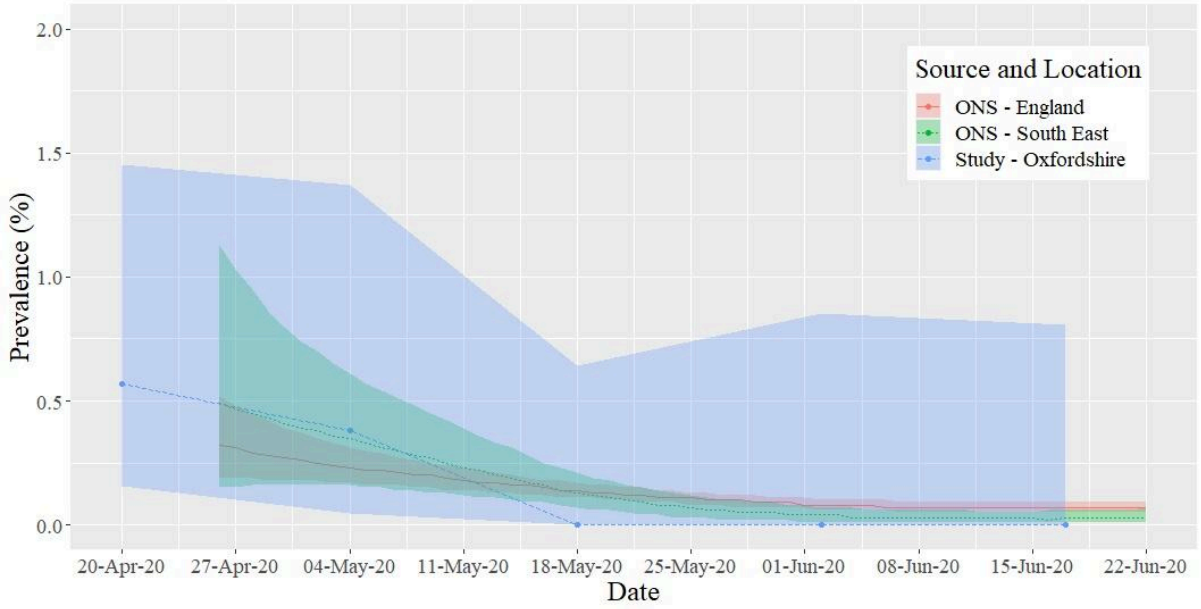


Figure 4.3 Prevalence of SARS-CoV-2 infection in the community as detected using RT-PCR as a part of this study in Oxfordshire (blue) compared with published modelled prevalence data [258] for all of England (red) and the South East region (green) with 95% binomial confidence intervals. ONS: Office for National Statistics; RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

For all participants with valid results, RT-PCR positive vs negative individuals were compared in Tables 4.10 and 4.11. Out of the six positive on RT-PCR for SARS-CoV-2, four (66.7%) were male, with ages 20 - 69 years with a median of 29 years (IQR: 25 - 39 years). Two (33.3%) of the RT-PCR positive participants met the symptom-based case definition compared with 71/2590 (2.7%) RT-PCR negative participants. Using Fisher's exact test for count data, this gives an odds ratio (OR) with (95% CI) of 17.7 (1.6 - 125.2) of RT-PCR positive participants meeting the symptom-based case definition criteria vs RT-PCR negative participants and *p*-value of 0.011. Three (50.0%) RT-PCR positive participants had positive serology vs 137/2063 (6.6%) RT-PCR negative participants for which valid results were available, giving an OR (95%) of 14.0 (1.9 - 105.8) and *p*-value of 0.0052. Other variables examined did not show significant differences between RT-PCR positive and negative participants.

Table 4.10 Comparison of SARS-CoV-2 RT-PCR positive vs negative participants using study variables. Only participants with valid results are included, with the relevant subpopulations for each section in **bold**. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Variable	RT-PCR Positive	RT-PCR Negative	*p*-value*
	n (%)	n (%)	
Participants	**n = 6**	**n= 2678**	
Sex			
Female	2 (33.3)	1449 (54.1)	0.42
Male	4 (66.6)	1229 (45.9)	
Age Group (years)			
18 - 29	3 (50.0)	824 (30.8)	0.45
30 - 44	2 (33.3)	722 (27.0)	
45 - 64	0 (0.0)	718 (26.8)	
65+	1 (16.7)	414 (15.5)	
Round			
1	4 (66.7)	698 (26.1)	0.11
2	2 (33.3)	523 (19.5)	
3	0 (0.0)	572 (21.4)	
4	0 (0.0)	430 (16.1)	
5	0 (0.0)	455 (17.0)	
Responding participants	**n = 6**	**n= 2590**	
Household size			
1	0 (0.0)	178 (68.7)	0.60
2	3 (50.0)	1013 (39.1)	
3	1 (16.7)	484 (18.7)	
4	0 (0.0)	531 (20.5)	
5+	2 (33.3)	384 (14.8)	
Key Worker Status			
Yes	1 (16.7)	475 (18.3)	0.11
No	4 (66.7)	2073 (80.0)	
Unknown	1 (16.7)	42 (1.6)	
Economic Activity			
Paid Employment	3 (50.0)	1524 (48.8)	0.52
Self-employed	0 (0.0)	233 (9.0)	
Student	2 (33.3)	300 (11.6)	
Retired	1 (16.7)	385 (14.9)	
Other	0 (0.0)	148 (5.7)	
Occupation			
Health or social care	1 (16.7)	227 (8.8)	0.50
Professional	1 (16.7)	978 (37.8)	
Business or administration	1 (16.7)	317 (12.2)	
Other	0 (0.0)	232 (9.0)	
None	3 (50.0)	836 (32.3)	
Highest level of education obtained			
O-levels or equivalent	0 (0.0)	101 (3.9)	0.34
A-levels or equivalent	1 (16.7)	266 (10.3)	
Bachelor’s or equivalent	0 (0.0)	782 (30.2)	
Higher degree	5 (83.3)	1414 (54.6)	
None of the above	0 (0.0)	27 (1.0)	

*The *p*-value is calculated using Fisher's exact test for count data for all 2x2 cases. For all instances larger than 2x2, the Freeman-Halton extension of Fisher's exact test is used.

Table 4.11 Comparison of SARS-CoV-2 RT-PCR positive vs negative participants using study variables. Only participants with valid results are included, with the relevant subpopulations for each section in **bold**. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Variable	RT-PCR Positive n (%)	RT-PCR Negative n (%)	*p*-value*
Responding participants	**n = 6**	**n= 2590**	
Chronic medical condition			
Yes	2 (33.3)	586 (23.6)	0.63
No	4 (66.7)	1898 (76.4)	
Current nicotine use			
Yes	0 (0.0)	208 (8.0)	1
No	6 (100.0)	2382 (92.0)	
Chronic respiratory allergies			
Yes	5 (83.3)	1227 (47.4)	0.11
No	1 (16.7)	1363 (52.6)	
Any current acute symptom			
Yes	2 (33.3)	443 (17.1)	0.27
No	4 (66.7)	2147 (82.9)	
Social distancing			
Yes	6 (100.0)	1705 (65.8)	0.10
No	0 (0.0)	885 (34.2)	
Social isolation			
Yes	0 (0.0)	130 (5.0)	1
No	6 (100.0)	2460 (95.0)	
Symptom-based case definition			
Positive	2 (33.3)	71 (2.7)	0.011
Negative	4 (66.7)	2519 (97.3)	
Sequenced case-control participants	**n = 6**	**n= 24**	
Metagenomic next-generation sequencing			
Positive	2 (33.3)	1 (4.2)	0.094
Negative	4 (66.7)	23 (95.8)	
Participants with valid serology results	**n = 6**	**n= 2590**	
Specific serology			
IgM Positive	0 (0.0)	31 (1.5)	0.0024
IgG Positive	3 (50.0)	69 (3.3)	
IgM and IgG Positive	0 (0.0)	37 (1.8)	
Negative	3 (50.0)	1926 (93.4)	
Any serology			
Positive	3 (50.0)	137 (6.6)	0.0052
Negative	3 (50.0)	1926 (93.4)	

*The *p*-value is calculated using Fisher's exact test for count data for all 2x2 cases. For all instances larger than 2x2, the Freeman-Halton extension of Fisher's exact test is used.

4.4.2.2 Symptom-based Case Definition

A total of 74 participants met one or more symptom-based case definition criteria, with a decreasing trend from Round 1 to 5, as was seen in Table 4.7. The most common qualifying symptom was the presence of a new acute cough (n = 69) followed by loss of smell (n = 8) or

taste (n = 4), with fever reported in three participants. Of the six RT-PCR positive participants, two (33.3%) had documented any COVID-19 symptoms, as listed in Table 4.6, including those in the symptom-based case definition criteria. One 69-year-old male reported cough, sore throat, shortness of breath, headache, tiredness and loss of smell. The other symptomatic participant was a 24-year-old male with a sore throat and cough. The remaining four RT-PCR positive participants (66.7%) were asymptomatic at time of testing.

The symptom-based case definition was compared against RT-PCR as the gold standard, as shown in Table 4.12 for all participants with valid results. Compared with RT-PCR, the symptom-based case definition had a sensitivity (95% CI) of 33.3% (4.3 - 77.7%) and specificity of 97.3% (96.6 - 97.9%). The number of false positives was relatively high, with an FDR of 97.3% (90.5 - 99.7%). The overall accuracy of the symptom-based case definition was 97.1% (96.4 - 97.7%) compared with RT-PCR at this prevalence level. When examining measures independent of the study prevalence, it was found that the LR+ (95% CI) was 12.2 (3.8 - 38.6). This result indicates that meeting the symptom-based case definition is strongly associated with being truly positive for COVID-19 vs negative as determined by RT-PCR, with the lower bound of the 95% CI not crossing the likelihood ratio threshold of 1.0. The LR- was 0.69 (0.39 - 1.2), with the 95% CI indicating no significant difference in the probability of having COVID-19 vs not as determined by RT-PCR when the symptom-based case definition criteria are not met. Overall, the DOR was 17.7 (3.2 - 98.4), indicating high odds of meeting the symptom-based case definition in those with COVID-19 as determined by RT-PCR relative to the odds of meeting the criteria in those without the disease.

Table 4.12 Comparing the symptom-based case definition for SARS-CoV-2 against RT-PCR as the gold standard. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; Sn: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Ac: Accuracy; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; DOR: Diagnostic odds ratio; FDR: False discovery rate.

		Gold Standard Test for SARS-CoV-2 (RT-PCR)		FDR (95% CI) 97.3% (90.5 - 99.7%)	
		Positive	Negative		
Symptom-based Case Definition	Positive	2	71	PPV (95% CI) 2.7% (0.88 - 8.2%)	LR+ (95% CI) 12.2 (3.8 - 38.6)
	Negative	4	2519	NPV (95% CI) 99.8% (99.7 - 99.9%)	LR- (95% CI) 0.69 (0.39 - 1.2)
		Sn (95% CI) 33.3% (4.3 - 77.7%)	Sp (95% CI) 97.3% (96.6 - 97.9%)	Ac (95% CI) 97.1% (96.4 - 97.7%)	DOR (95% CI) 17.7 (3.2 - 98.4)

4.4.2.3 Serology

Of the 2,140 participants with complete and valid serology results, 146 individuals (6.8%) had SARS-CoV-2 antibodies detected. After accounting for any effects of clustering in same-household participants using a GEE model, the overall seroprevalence (95% Wald CI) was 6.6% (5.6 - 7.8%). In total, there were 31/2140 (1.4%) individuals with IgM-positive only, 39 (1.8%) with IgM and IgG-positive and 76 (3.6%) with IgG-positive only. The four serology brands used in the study were compared in Table 4.13. This table includes valid RT-PCR results; however, the timeframe for antibody positivity can extend beyond the RT-PCR detection window, as illustrated in Figure 4.1. Given this and the low cell counts, sensitivity and specificity were not calculated for individual serology results in this study. Instead, sensitivity and specificity estimates from the literature for each serology brand and

specific antibody response were included, as compared against SARS-CoV-2 immunoassay reference panels. Sensitivity and specificity estimates varied by brand, with the lowest sensitivity being 14.3% for Vazyme® IgM-positive only.

Table 4.13 Comparison of the four serology brands used in the study against RT-PCR results by Round, with sensitivity and specificity measures obtained from the literature. RT-PCR: reverse-transcription polymerase chain reaction; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Serology Brand	Valid Serology Result	Valid RT-PCR result		Sensitivity	Specificity	Reference
		Positive n (%)	Negative n (%)	% (95% CI)	% (95% CI)	
VivaDiag®	Round 1 (n = 264)			Estimate from the literature		259
	IgM	0 (0.0)	3 (1.1)	65.4 (57.5 – 72.5)	100 (95.7 – 100)	
	IgG	0 (0.0)	2 (0.76)	62.8 (54.9 – 70.0)	99.0 (94.2 – 100)	
	IgM + IgG	0 (0.0)	18 (6.8)	62.8 (54.9 – 70.0)	97.1 (91.4 – 99.4)	
	Negative	3 (1.1)	238 (90.2)			
Orient Gene®	Round 1 (n = 380)			Estimate from the literature		259
	IgM	0 (0.0)	4 (1.1)	72.5 (65.0 – 79.0)	95.1 (88.9 – 98.2)	
	IgG	1 (0.26)	6 (1.6)	68.0 (60.2 – 74.9)	93.2 (86.4 – 96.9)	
	IgM + IgG	0 (0.0)	6 (1.6)	64.1 (56.2 – 71.2)	100 (95.7 – 100)	
	Negative	0 (0.0)	363 (95.5)			
Vazyme®	Round 2 (n = 483)			Estimate from the literature		260
	IgM	0 (0.0)	10 (2.1)	14.3 (6.8 – 27.6)	81.3 (67.1 – 90.2)	
	IgG	2 (0.41)	18 (3.7)	100 (92.9 – 100)	95.9 (84.4 – 99.0)	
	IgM + IgG	0 (0.0)	6 (1.2)	100 (92.9 – 100)	80.0 (66.1 – 89.1)	
	Negative	0 (0.0)	447 (92.5)			
	Round 3 (n = 146)			Estimate from the literature		260
	IgM	0 (0.0)	7 (4.8)	14.3 (6.8 – 27.6)	81.3 (67.1 – 90.2)	
	IgG	0 (0.0)	7 (4.8)	100 (92.9 – 100)	95.9 (84.4 – 99.0)	
	IgM + IgG	0 (0.0)	0 (0.0)	100 (92.9 – 100)	80.0 (66.1 – 89.1)	
	Negative	0 (0.0)	132 (90.4)			
CTK Biotech OnSite®	Round 4 (n = 397)			Estimate from the literature		261
	IgM	0 (0.0)	5 (1.3)	67.0 (56.9 – 76.1)	87.8 (79.6 – 93.5)	
	IgG	0 (0.0)	22 (5.5)	51.0 (40.8 – 61.1)	99.0 (94.5 – 100)	
	IgM + IgG	0 (0.0)	5 (1.3)	70.0 (60.0 – 78.8)	86.7 (78.4 – 92.7)	
	Negative	0 (0.0)	365 (91.9)			
	Round 5 (n = 396)			Estimate from the literature		261
	IgM	0 (0.0)	2 (0.51)	67.0 (56.9 – 76.1)	87.8 (79.6 – 93.5)	
	IgG	0 (0.0)	14 (3.5)	51.0 (40.8 – 61.1)	99.0 (94.5 – 100)	
	IgM + IgG	0 (0.0)	2 (0.51)	70.0 (60.0 – 78.8)	86.7 (78.4 – 92.7)	
	Negative	0 (0.0)	378 (95.5)			

Excluding participants who did not report any serology result and those in Round 3 who received LCB, the percentage of participants with invalid results varied by serology brand was: 2/273 (0.73%) for VivaDiag®, 12/842 (1.4%) for CTK Biotech OnSite®, 13/405 (3.2%) for Orient Gene® and 42/689 (6.1%) for Vazyme® tests. Three out of six (50.0%) RT-PCR positive participants had positive serology, all of which were IgG-positive only, with one obtained by Orient Gene® and the other two by Vazyme®. The remaining three RT-PCR positive participants were negative on VivaDiag® serology testing.

As was seen in Table 4.8, CTK Biotech OnSite® and Orient Gene® branded serology tests were both negative predictors of evidence of current or previous SARS-CoV-2 infection, with unadjusted ORs (95%CI) of 0.50 (0.33 - 0.77) and 0.58 (0.36 - 0.95) respectively, as compared to VivaDiag® as the reference group. The adjusted multiple regression analysis did not include serology brands because of the high multicollinearity with Round dates. For CTK Biotech OnSite®, the unadjusted OR result may be explained by its use in Round 5, which had lower evidence of current or previous SARS-CoV-2 infection in this different geographical region compared with Rounds 1 - 4, and thus the other serology brands. However, Orient Gene® was used alongside VivaDiag® in Round 1, suggesting that either Orient Gene® had a higher rate of false negatives or that VivaDiag® had a higher rate of false positives. This could not be further determined without access to SARS-CoV-2 immunoassay reference panels, and the overlap of the confidence intervals for sensitivity and specificity for VivaDiag® and Orient Gene® found in the literature and seen in Table 4.13 suggest no significant difference.

It was hypothesised that the participants from the same household (housemates) of those who tested positive for SARS-CoV-2 on RT-PCR would differ in serology positivity (IgG, IgM or

both) compared with non-housemates, with the H_A being that the true OR ≠ 1 given exposure. The H_0 was that there was no difference in serology results between the groups (true OR = 1). Excluding RT-PCR positive participants and those without valid results, a 2x2 table was constructed in Table 4.14 to perform Fisher's exact test, comparing the housemates of RT-PCR positive participants and those from other households. The OR (95% CI) of having any positive serology in housemates compared to non-housemates was 7.0 (0.63 - 49.6) with a *p*-value of 0.056. While the *p*-value was above the threshold for statistical significance defined in our methods, the observed OR suggests a difference in the odds of positive serology in housemates of RT-PCR positive individuals compared with non-housemates, even though the wide CI and low count in some cells indicate likely underpowering and calls for caution in interpretation.

Table 4.14 Comparison of housemates of SARS-CoV-2 RT-PCR positive individuals with participants from other households. SARS-CoV-2 RT-PCR positive participants and those with invalid serology results were excluded. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Variable	Serology Positive	Serology Negative	*p*-value*
	n (%)	n (%)	
Participants	**n = 143**	**n= 1991**	
Housemate of RT-PCR-positive participant	2 (1.4)	4 (0.20)	0.056
Other Households	141 (98.6)	1987 (99.8)	

*The *p*-value is calculated using Fisher's exact test for count data.

Results for any serology (IgM, IgG or both) were compared against RT-PCR, as shown in Table 4.15 for participants with valid results reported. The sensitivity (95% CI) of any positive serology was 50.0% (11.8 - 88.2%) with a specificity of 93.4% (92.2 - 94.4%). The PPV was low, with a high FDR of 97.9% (93.9 - 99.6%). The LR+ results indicated that positive serology was strongly associated with being positive for SARS-CoV-2 vs negative on RT-PCR. In contrast, the LR- (95% CI) of 0.54 (0.24 - 1.2) suggested negative serology results were not significantly associated with a reduced probability of having vs not having

COVID-19 as determined by RT-PCR. The DOR for serology was 14.1 with a 95%CI of 2.8 - 70.3, indicating high odds of positive serology in those with COVID-19 on RT-PCR relative to the odds of positive serology in those without the disease.

Table 4.15 Comparing any SARS-CoV-2 serology (IgG, IgM or both) against RT-PCR results as the gold standard. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IgG: Immunoglobulin G; IgM: Immunoglobulin M; Sn: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Ac: Accuracy; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; DOR: Diagnostic odds ratio; FDR: False discovery rate.

		Gold Standard Test for SARS-CoV-2 (RT-PCR)		FDR (95% CI) 97.9% (93.9 - 99.6%)	
		Positive	Negative		
Any Serology (IgG, IgM or both)	Positive	3	137	PPV (95% CI) 2.1% (0.96 - 4.7%)	LR+ (95% CI) 7.5 (3.3 - 17.0)
	Negative	3	1926	NPV (95% CI) 99.8% (99.7 - 99.9%)	LR- (95% CI) 0.54 (0.24 - 1.2)
		Sn (95% CI) 50.0% (11.8 - 88.2%)	Sp (95% CI) 93.4% (92.2 - 94.4%)	Ac (95% CI) 93.2% (92.1 - 94.3%)	DOR (95% CI) 14.1 (2.8 - 70.3)

4.4.2.4 Metagenomic Next-Generation Sequencing

Of the 116 participants who underwent sequencing, 62 (53.4%) were male with ages ranging 19 - 82 years with a median 37 years (IQR: 27 - 53 years). The median number of total reads obtained per sequenced participant was 44,972 reads (IQR: 16,953 - 109,256 reads). The median number of unclassified reads unable to be assigned a taxon ranking was 14,608 reads (IQR: 8,102 - 24,155 reads) with a median of 39.0% (IQR: 13.3 - 78.0%) unclassified reads per sequenced participant.

Eight participants had at least one read assigned to SARS-CoV-2 with a median of 43 reads (IQR: 19 - 194 reads) per positive participant. This finding implied a median relative abundance of SARS-CoV-2 of 0.31% (IQR: 0.073 - 0.78%) in the sequenced samples. Five (62.5%) of these participants were male with ages ranging from 20 - 56 years. Using Fisher's exact test for count data, 2/6 (33.3%) of the RT-PCR positive participants had reads assigned to SARS-CoV-2 on mNGS, compared with 1/24 (4.2%) of matched controls, giving a two-sided *p*-value of 0.094. The mNGS positive control only had a single read taxonomically assigned to SARS-CoV-2, which may suggest barcode crossover.[236] A total of 2/2 (100%) of the RT-PCR positive participants with intermediate C_T values had SARS-CoV-2 detected on mNGS, while 0/4 (0%) of those with high C_T values had reads detected. All seven household contacts of RT-PCR positive participants and the three individuals who had indeterminate RT-PCR results had zero SARS-CoV-2 reads detected on sequencing.

Table 4.16 compares mNGS results with RT-PCR as the gold standard for all sequenced case-control participants. The sensitivity of mNGS was 33.3% (4.3 - 77.7%) with a specificity of 95.8% (78.9 - 99.9%) compared with RT-PCR. The FDR of 33.3% (0.84 - 90.6%) was lower than for other tests, but with a wider confidence interval due to the smaller sample size. The LR+ and LR- results indicated that mNGS positive and negative results were not strongly associated with having or not having COVID-19 as confirmed by RT-PCR. The DOR (95% CI) for mNGS was 11.5 (0.83 - 158.7). This suggests an elevated likelihood of mNGS positivity in those with SARS-CoV-2 detected on RT-PCR compared to those without. Nevertheless, due to the wide confidence interval that includes the threshold value of 1.0, caution should be exercised in interpreting this as conclusive due to likely underpowering of the study.

Table 4.16 Comparing metagenomic next-generation sequencing against RT-PCR results as the gold standard. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; mNGS: metagenomic next-generation sequencing; Sn: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Ac: Accuracy; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; DOR: Diagnostic odds ratio; FDR: False discovery rate.

		Gold Standard Test for SARS-CoV-2 (RT-PCR)		FDR (95% CI) 33.3% (0.84 - 90.6%)	
		Positive	Negative		
mNGS	Positive	2	1	PPV (95% CI) 66.7% (17.7 - 94.9%)	LR+ (95% CI) 8.0 (0.86 - 74.2)
	Negative	4	23	NPV (95% CI) 85.2% (76.5 - 91.1%)	LR- (95% CI) 0.70 (0.39 - 1.2)
		Sn (95% CI) 33.3% (4.3 - 77.7%)	Sp (95% CI) 95.8% (78.9 - 99.9%)	Ac (95% CI) 83.3% (65.3 - 94.4%)	DOR (95% CI) 11.5 (0.83 - 158.7)

4.4.3 Microbiome Analysis

Participants with mNGS data available underwent microbiome analysis. First, a visual inspection of the microbiome composition was undertaken. Figure 4.4 shows a Krona metagenomic visualisation via the BugSeq platform of the participant with the highest SARS-CoV-2 read count using described methods.[262] This participant had 16,906 reads assigned to SARS-CoV-2, accounting for 22.6% of all reads in the sample. Excluding unclassified reads, this participant's second-highest read count was assigned to *Haemophilus parainfluenzae* at the species level.

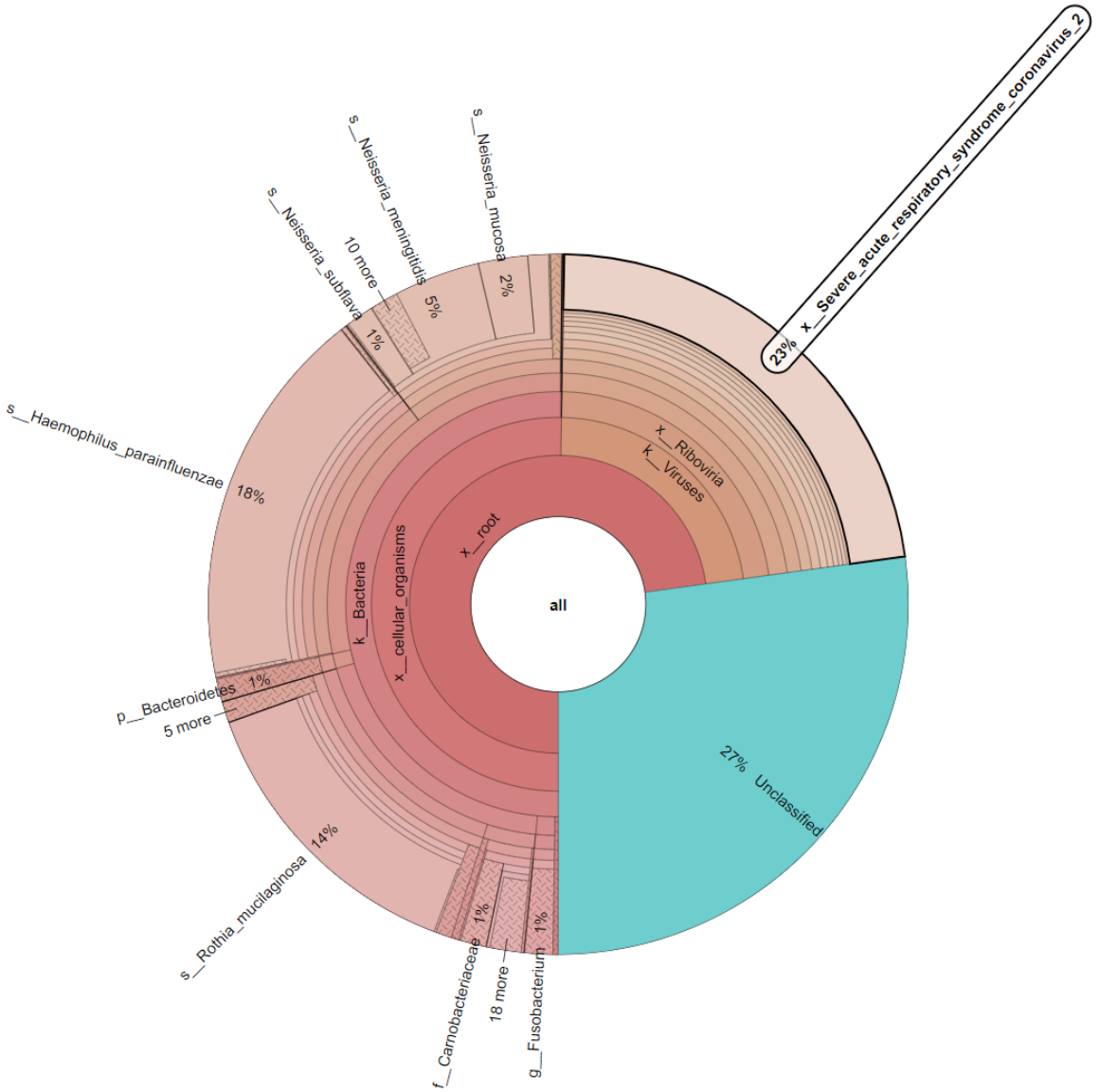


Figure 4.4 Krona metagenomic visualisation of the sequenced reads from one participant who had a SARS-CoV-2 read count of 16,906 reads. The black box indicates the reads assigned to SARS-CoV-2. This pie chart was generated via BugSeq using described methods.[262] SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

4.4.3.1 Relative Abundance

The relative abundance of species with sequenced reads assigned at this taxon level for RT-PCR positive cases (n = 6) vs their negative controls (n = 24) is shown in Figure 4.5. Unclassified reads comprised most of the samples for both groups. The top four species for both RT-PCR positive and controls were the same, but RT-PCR positive participants had a

greater relative abundance of *Haemophilus parainfluenzae*. However, on comparing the relative abundance of this species between cases vs control using the non-parametric Wilcoxon test, the *p*-value was 0.34, indicating no significant difference between the groups.

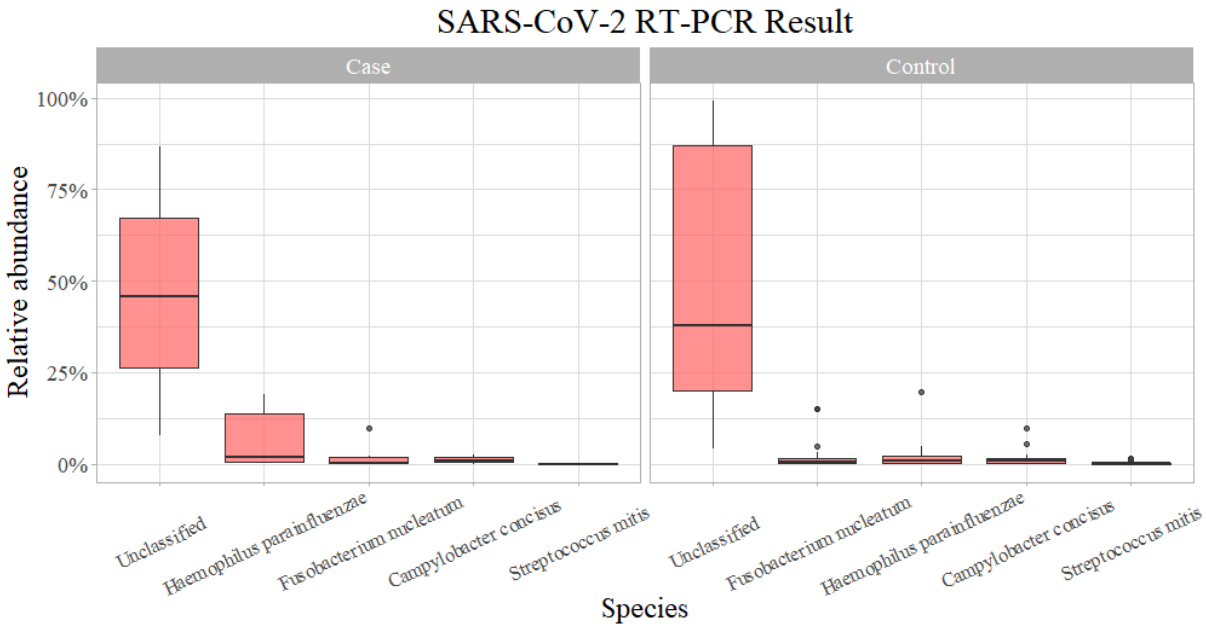


Figure 4.5 Top relative abundance of sequenced reads in SARS-CoV-2 RT-PCR positive cases (n = 6) vs their negative controls (n = 24) assigned at the species level. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

The relative abundance of sequenced reads was explored at different taxonomy levels. Figure 4.6 shows reads at the phylum level for RT-PCR positive participants vs their negative controls. Again, unclassified reads comprised the majority of the relative abundance for both. The top phylum for both RT-PCR cases and controls were the same, but RT-PCR positive participants had a greater relative abundance of Proteobacteria than controls. Proteobacteria is the phylum which contains *Haemophilus parainfluenzae*, amongst other species. The relative abundance of proteobacteria was compared between cases vs control using the non-parametric Wilcoxon test, giving a *p*-value was 0.14, indicating no significant difference between the groups.

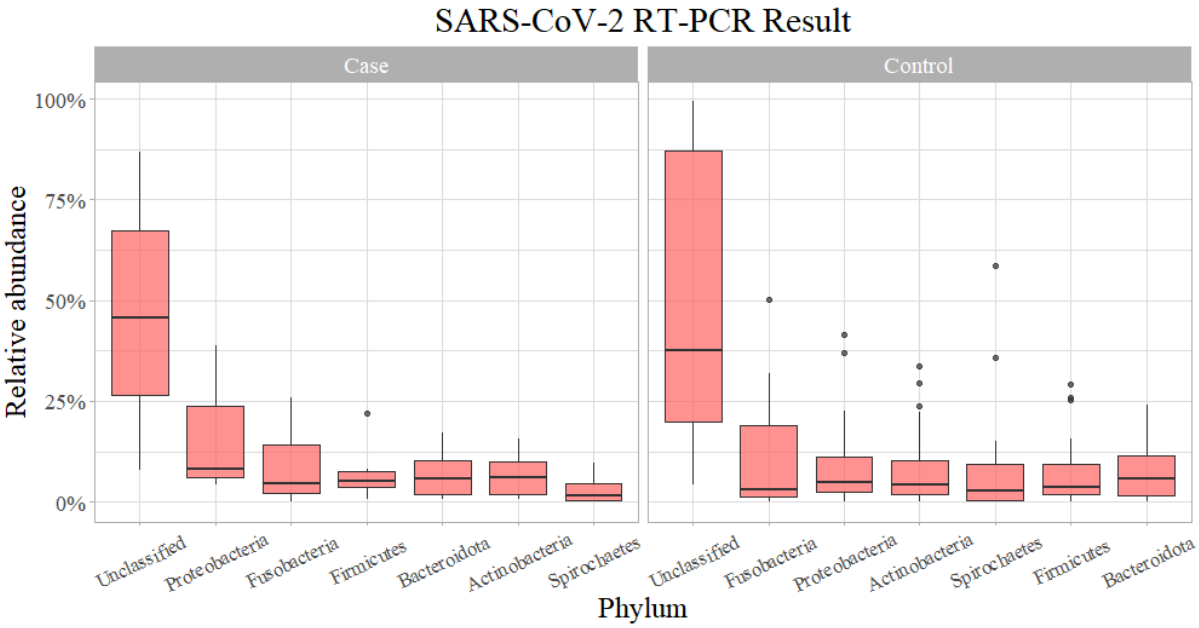


Figure 4.6 Top relative abundance of sequenced reads in SARS-CoV-2 RT-PCR positive cases (n = 6) vs their negative controls (n = 24) assigned at the phylum level. RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

4.4.3.2 Alpha Diversity

Alpha diversity was explored at the species level for RT-PCR positive participants vs their negative controls, as shown in Figure 4.7. RT-PCR positive cases had similar Shannon and Simpson diversity index scores compared with controls, with no significant difference found between the groups (Wilcoxon test *p*-values 0.82 and 0.98, respectively). The count of the number of species, represented by richness, was similar between the two groups, with a *p*-value of 0.98. Except for a few outliers, the evenness or distribution of species abundance was similar between cases and controls, with a *p*-value of 0.86. Other taxonomy levels were also explored but did not demonstrate significant differences between the two groups using these four metrics for alpha diversity.

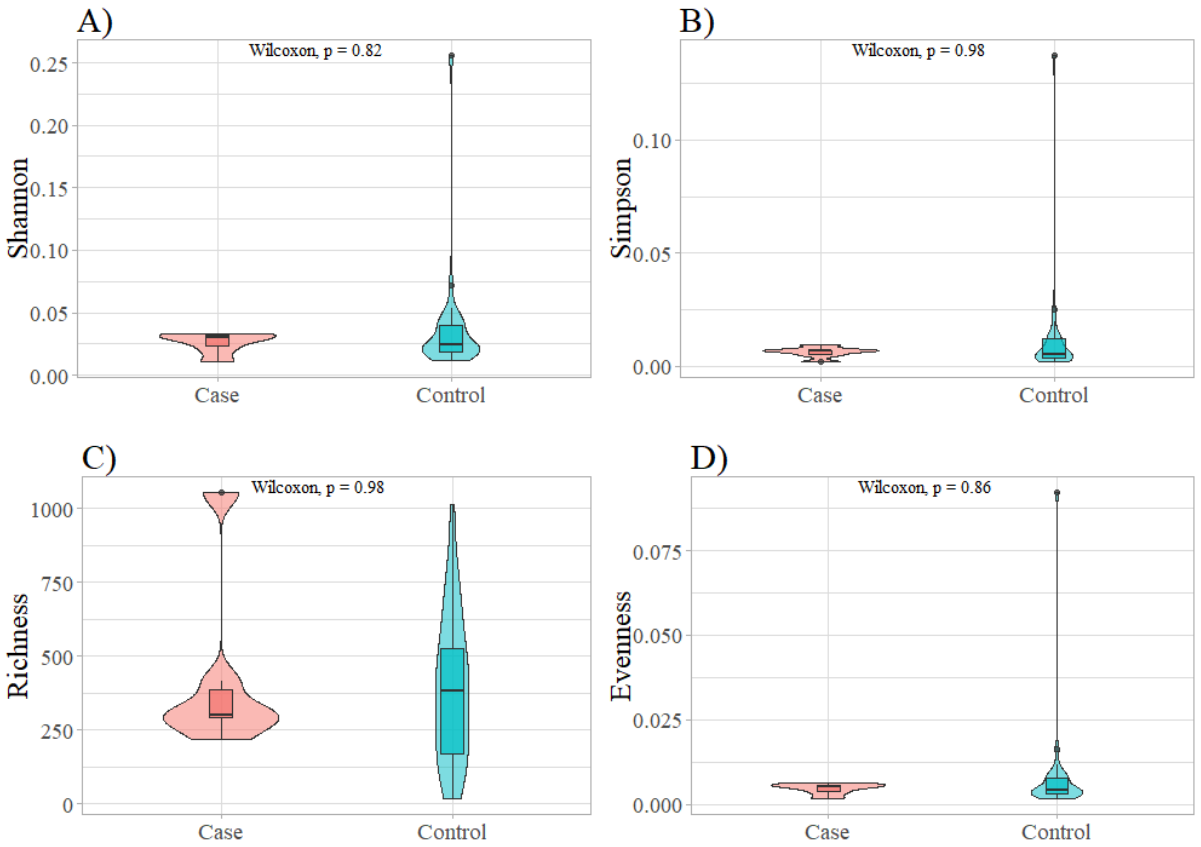


Figure 4.7 Alpha diversity at the species level comparing RT-PCR positive cases (n = 6) in red and their matched controls (n = 24) in blue. *P*-values were obtained by comparison of the two groups using the non-parametric Wilcoxon rank-sum test. A: Shannon diversity index; B: Simpson diversity index; C: Richness; D: Evenness based on Shannon diversity index.

Alpha diversity was compared at the genus level for sequenced participants who met the symptom-based case definition, as shown in Figure 4.8. Shannon and Simpson diversity index scores were similar between both groups (Wilcoxon test *p*-values 0.26 and 0.16, respectively), with more diverse outliers amongst those who met the symptom-based case definition. There was similar richness (*p*-value 0.16) and evenness (*p*-value 0.34) between the two groups, with more evenness amongst those not meeting the symptom-based case definition. Repeating these alpha diversity tests for different taxonomy levels did not produce statistically significant differences between the groups.

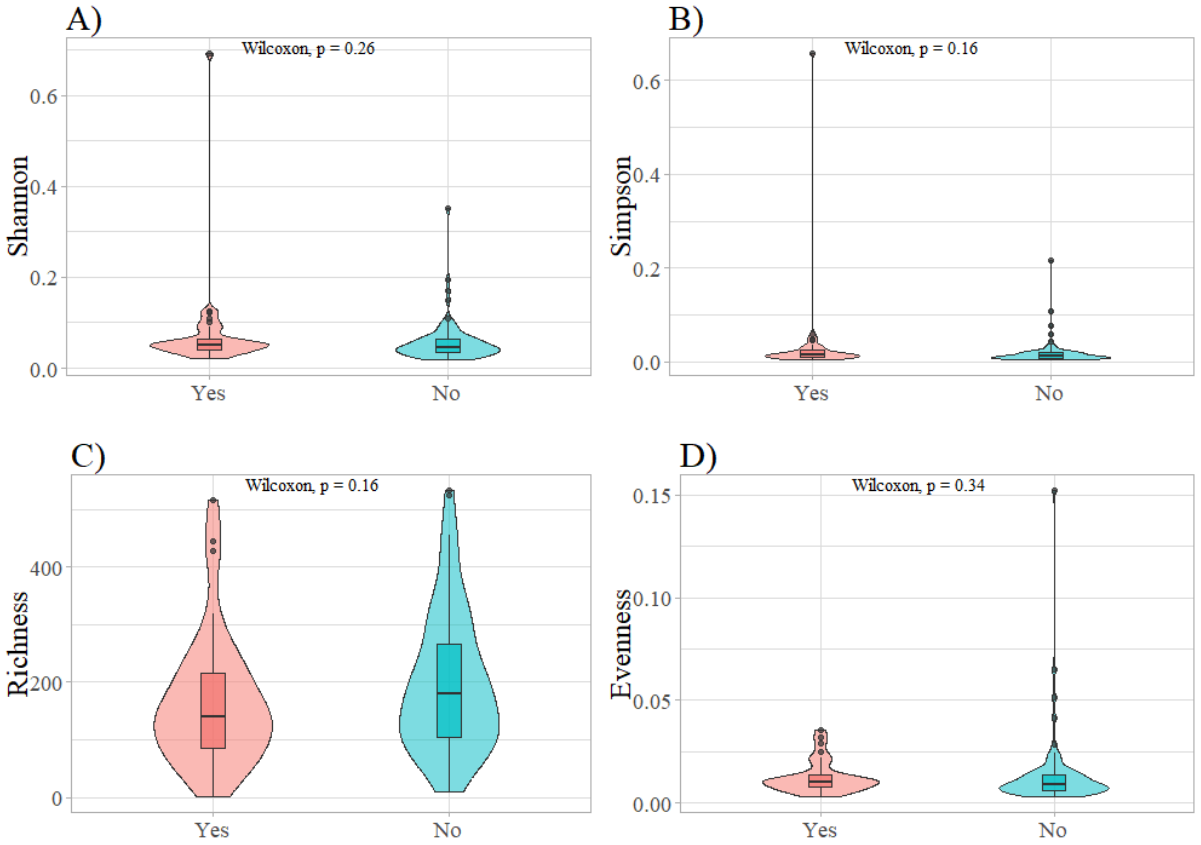


Figure 4.8 Alpha diversity at the genus level for those who met the symptom-based case definition (Yes, n = 37) in red and those who did not (No, n = 79) in blue. *P*-values obtained by comparison of the two groups using the non-parametric Wilcoxon rank-sum test. A: Shannon diversity index; B: Simpson diversity index; C: Richness; D: Evenness based on Shannon diversity index.

Alpha diversity was next compared at the family level for all sequenced participants with valid serology results, as shown in Figure 4.9. There was no significant difference in Shannon or Simpson diversity index scores (Kruskal-Wallis test *p*-values 0.29 and 0.77, respectively), with more diverse outliers at the family level in participants with negative serology, likely attributable to the larger sample size in this group. The richness and evenness were also similar between the four serology groups, with no significant difference found on statistical analysis (Kruskal-Wallis test *p*-values 0.78 and 0.39, respectively).

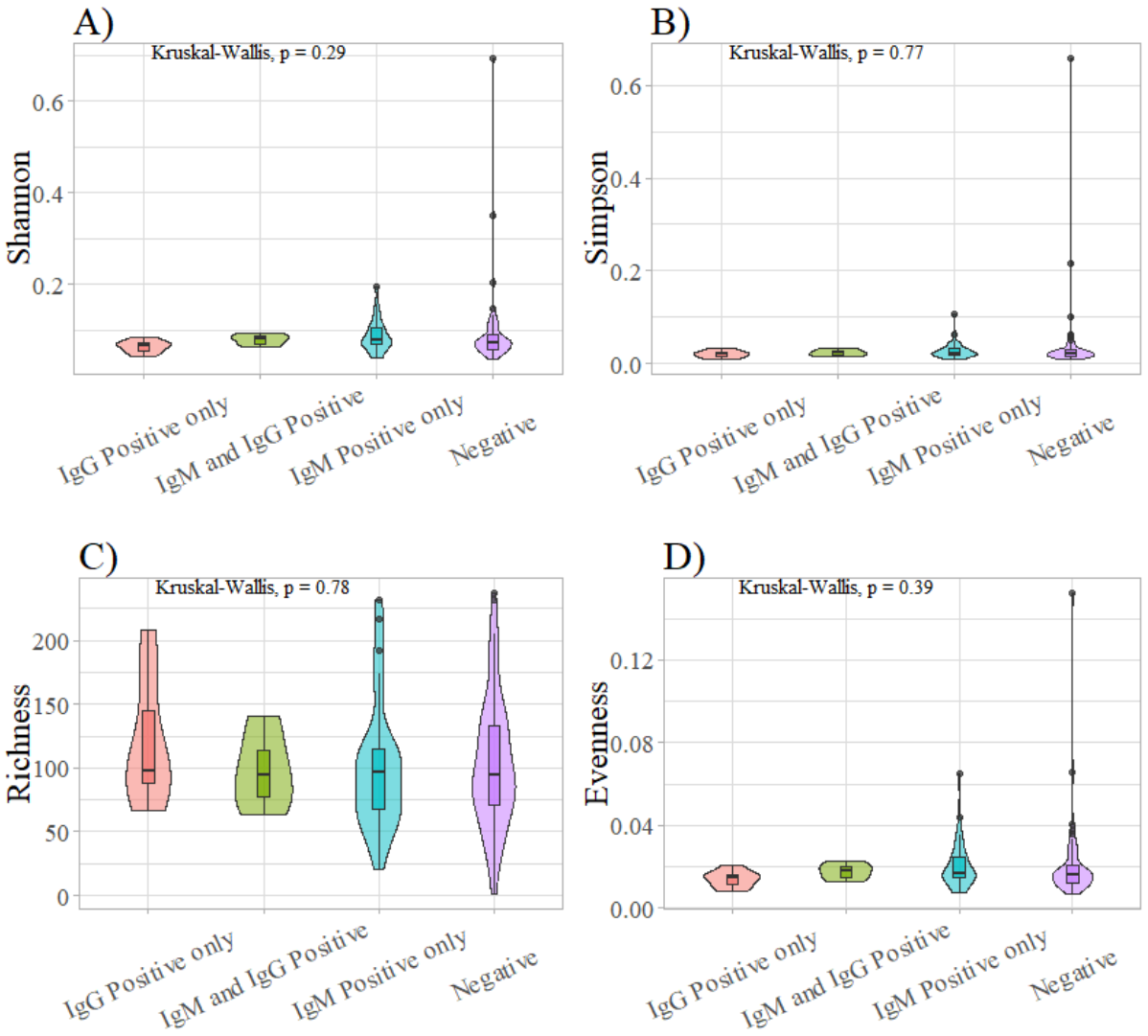


Figure 4.9 Alpha diversity at the family level for participants with valid serology results with IgG positive only (n= 7) in red, IgM and IgG positive (n = 5) in green, IgM positive only (n = 31) in blue and negative (n = 68) in purple. *P*-values were obtained by comparison of the four groups using the non-parametric Kruskal-Wallis test. A: Shannon diversity index; B: Simpson diversity index; C: Richness; D: Evenness based on Shannon diversity index; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Finally, alpha diversity was explored at the order level for any evidence of current or previous infection vs no evidence as shown in Figure 4.10. There was no significant difference in Shannon or Simpson diversity index scores between the two groups (Wilcoxon test *p*-values 0.22 and 0.20, respectively). The richness and evenness were also similar, with no significant difference found (Wilcoxon test *p*-values 0.19 and 0.26, respectively).

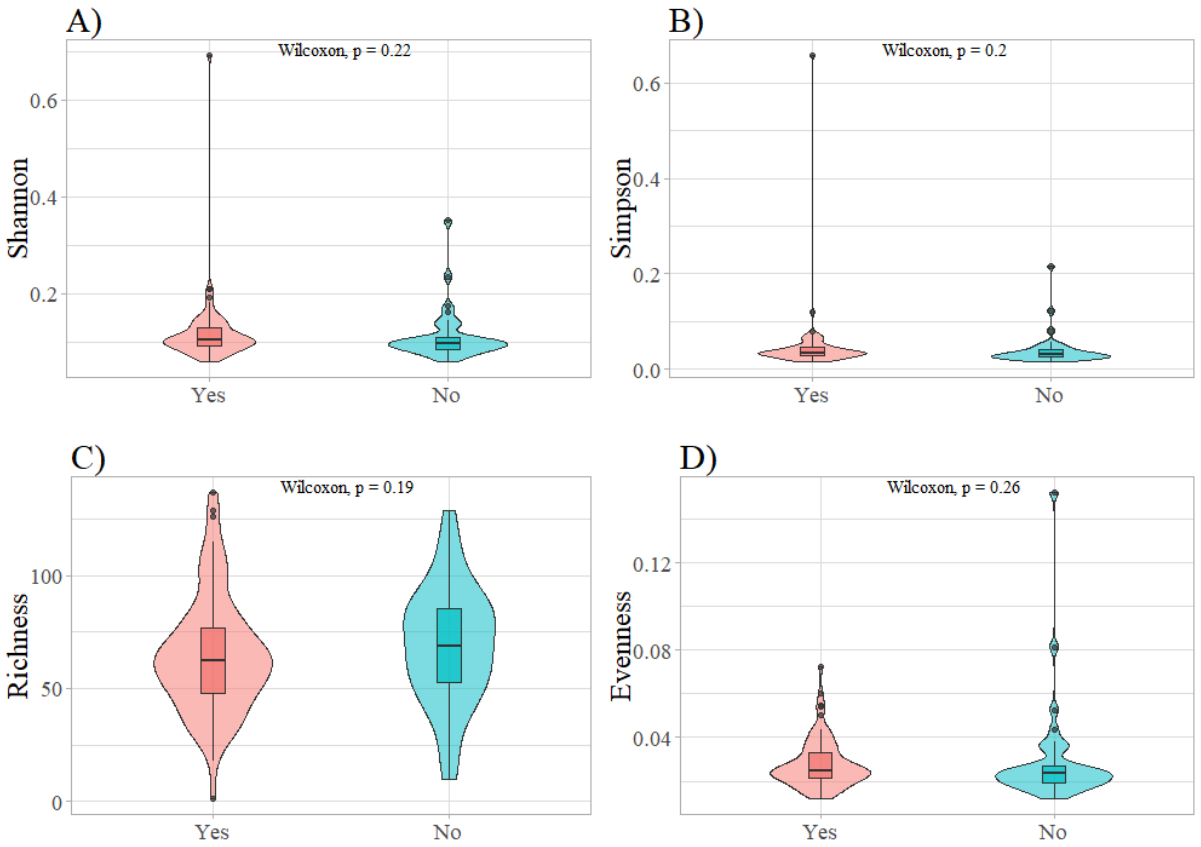


Figure 4.10 Alpha diversity at the order level for any evidence of current or past infection (Yes, n = 80) in red and those who did not (No, n = 36) in blue. *P*-values were obtained by comparison of the two groups using the non-parametric Wilcoxon rank-sum test. A: Shannon diversity index; B: Simpson diversity index; C: Richness; D: Evenness based on Shannon diversity index.

4.4.3.3 Beta Diversity

To explore beta diversity, a PCoA based on Bray-Curtis dissimilarity was conducted at the species level for RT-PCR positive cases (n = 6) vs matched negative controls (n = 24) as shown in Figure 4.11. The first axis explained 34.1% of the variance at the species level, while the second axis explained 14.2%. The PERMANOVA using distance matrices within the beta diversity matrix comparing cases vs controls returned a *p*-value of 0.84, indicating no significant difference. Repeating this analysis at different taxonomy levels returned no statistically significant differences between cases and control groups.

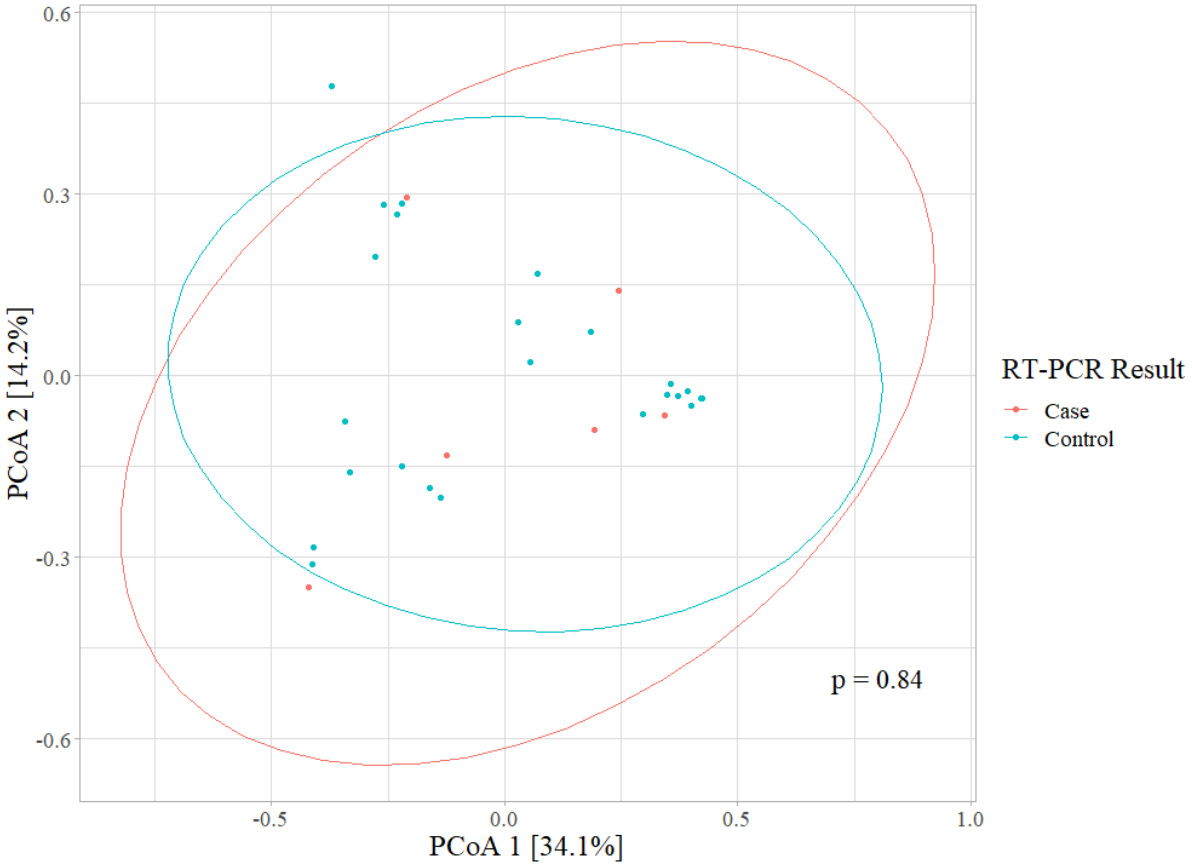


Figure 4.11 Principal coordinate analysis based on Bray-Curtis dissimilarity at the species level for RT-PCR positive cases (blue) vs their matched negative controls (red). Ellipses indicate the 95% confidence interval for each group. The square brackets indicate the percent of the variance explained by either axis while the *p*-value was obtained from the Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices comparing the two groups. RT-PCR: reverse-transcription polymerase chain reaction; PCoA: Principal coordinate analysis.

Next, a PCoA based on Bray-Curtis dissimilarity was conducted at the genus level for those who met the symptom-based case definition (n = 37) vs those who did not (n = 79) as shown in Figure 4.12. The first axis explained 26.8% of the variance at the genus level, while the second explained 9.0%. The PERMANOVA using distance matrices within the beta diversity matrix comparing the two groups returned a *p*-value of 0.055, indicating no significant difference. Repeat analyses at different taxonomy levels to compare those who met the symptom-based case definition vs those who did not return statistically insignificant differences.

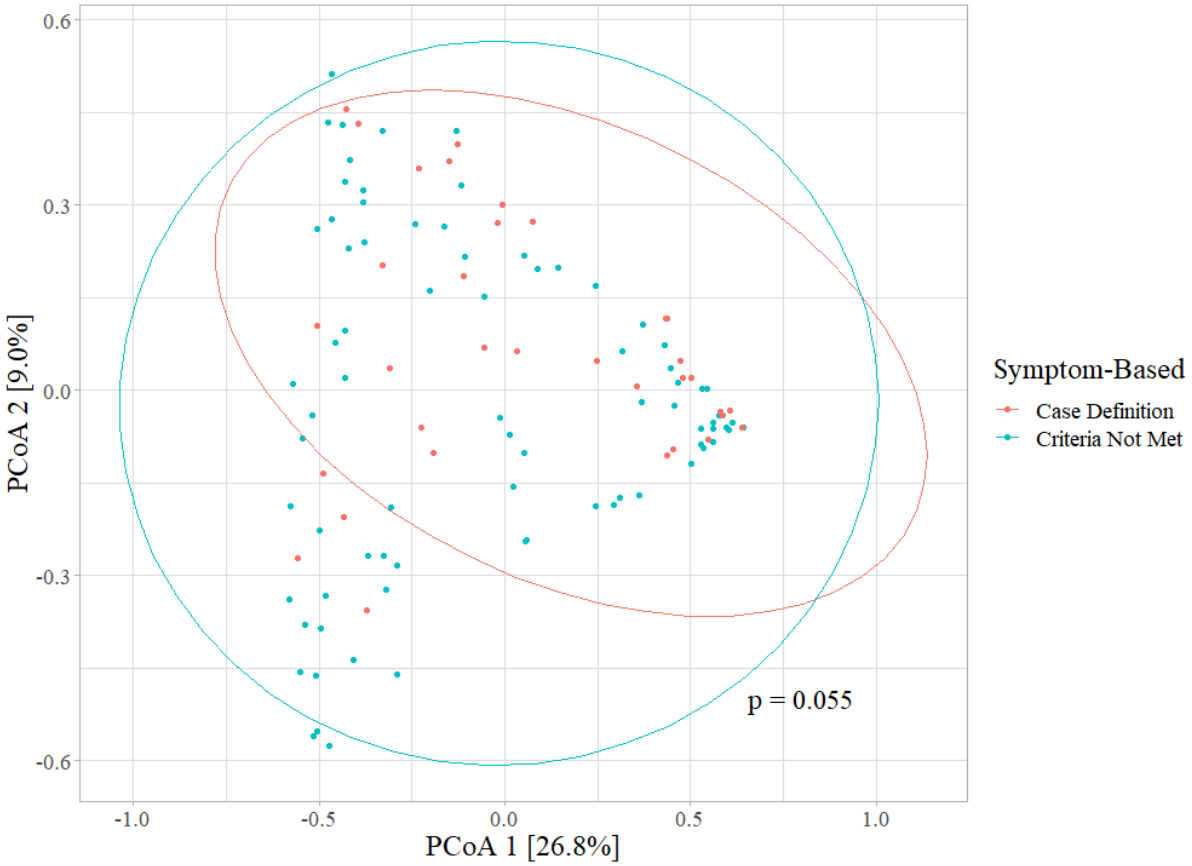


Figure 4.12 Principal coordinate analysis based on Bray-Curtis dissimilarity at the genus level for those who met the symptom-based case definition in red (n = 37) vs those who did not meet the criteria in blue (n = 79). Ellipses indicate the 95% confidence interval for each group. The square brackets indicate the percent of the variance explained by either axis, while the *p*-value was obtained from the Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices comparing the two groups. PCoA: Principal coordinate analysis.

A PCoA based on Bray-Curtis dissimilarity was conducted at the family level for those with valid serology results, as shown in Figure 4.13. The first axis explained 26.6% of the variance at the family level, while the second axis explained 9.5%. The PERMANOVA comparing the four groups returned a *p*-value of 0.44, indicating no significant difference. Repeating this analysis for any serology positive (n = 43) vs negative (n = 68) also showed no significant difference at the family level or for other taxonomy levels.

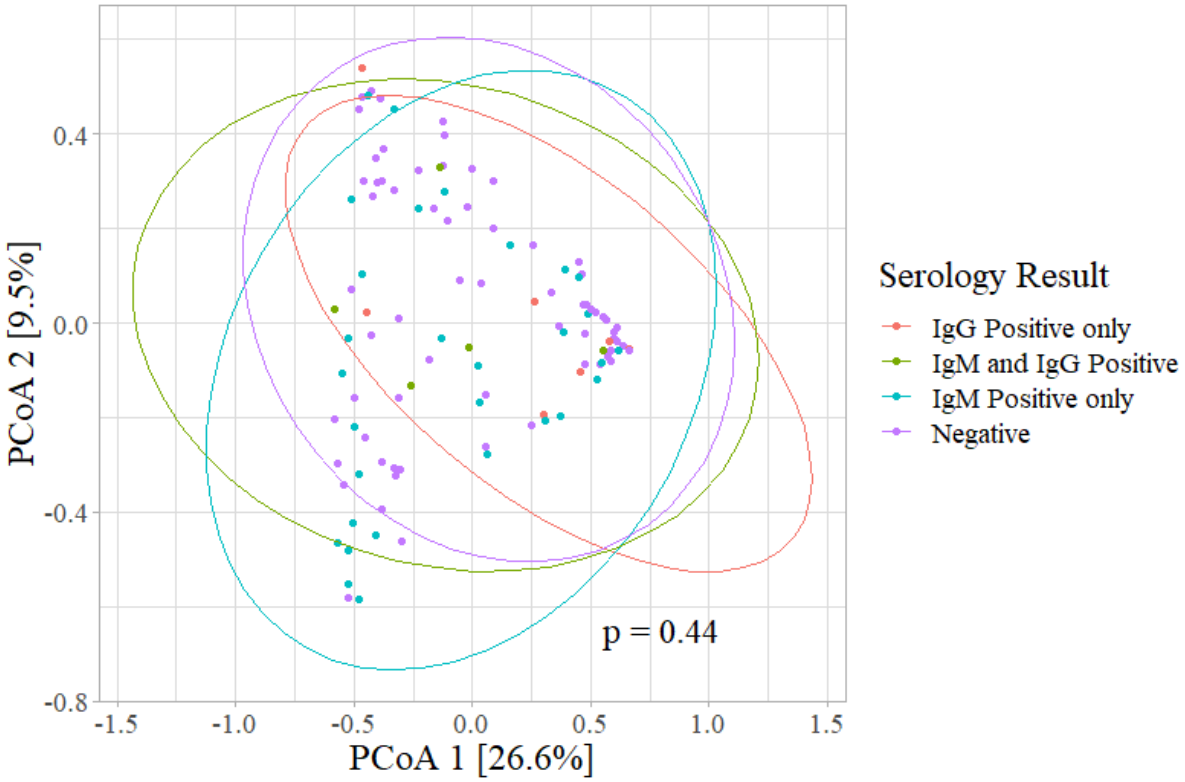


Figure 4.13 Principal coordinate analysis based on Bray-Curtis dissimilarity at the family level for those with valid serology results with IgG positive only (n= 7) in red, IgM and IgG positive (n = 5) in green, IgM positive only (n = 31) in blue and negative (n = 68) in purple. Ellipses indicate the 95% confidence interval for each group. The square brackets indicate the percent of the variance explained by either axis, while the *p*-value was obtained from the Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices comparing the four groups. PCoA: Principal coordinate analysis; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Finally, a PCoA based on Bray-Curtis dissimilarity was conducted at the order level to compare participants with any evidence of current or previous SARS-CoV-2 infection (n = 80) vs no evidence (n = 36) as shown in Figure 4.14. The first axis explained 28.7% of the variance at the order level, while the second explained 6.8%. The PERMANOVA comparing the two groups returned a *p*-value of 0.79, indicating no significant difference. Repeating this analysis at different taxonomy levels did not produce statistically significant results.

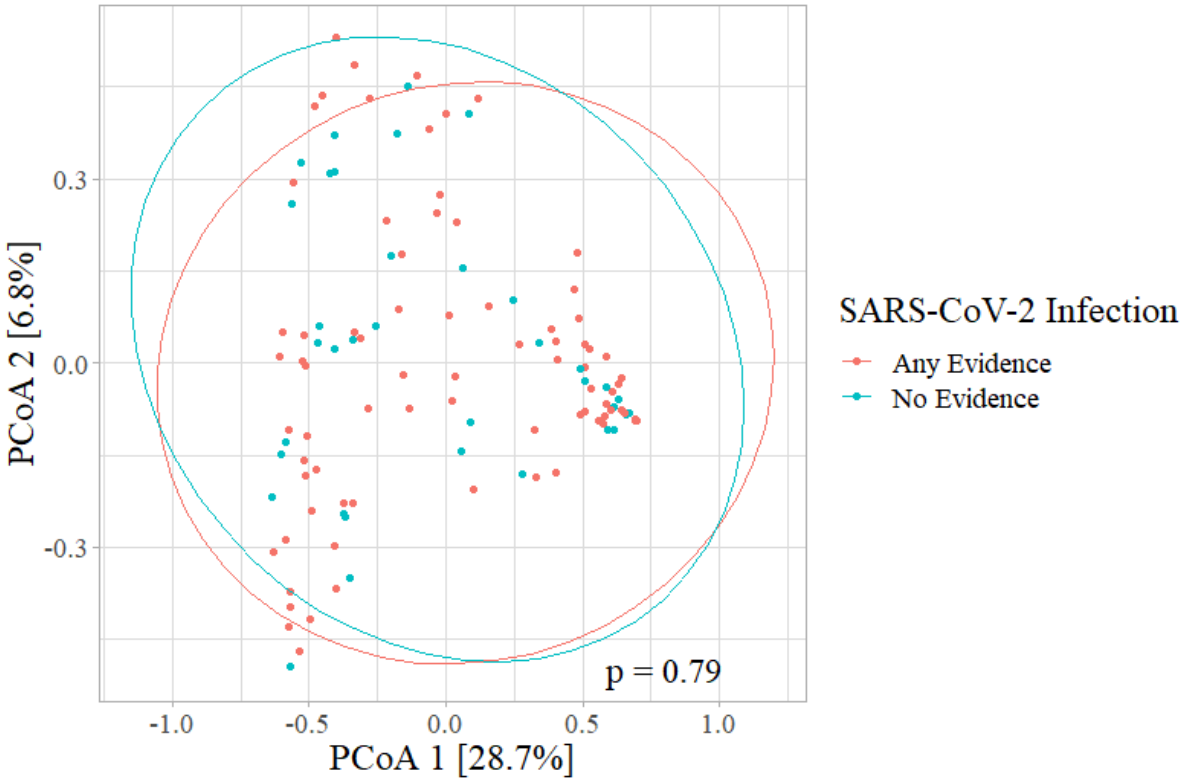


Figure 4.14 Principal coordinate analysis based on Bray-Curtis dissimilarity at the order level for participants with any evidence of current or previous SARS-CoV-2 infection (red) vs no evidence (blue). Ellipses indicate the 95% confidence interval for each group. The square brackets indicate the percent of the variance explained by either axis, while the *p*-value was obtained from the Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices comparing the two groups. PCoA: Principal coordinate analysis; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

4.4.3.4 Differential Abundance Analysis

A DAA was undertaken for all four groups under examination. Figure 4.15 shows volcano plots of the effect size vs the Kruskal-Wallis *p*-value and Benjamini-Hochberg corrected *q*-value for SARS-CoV-2 RT-PCR positive cases vs their negative controls. One OTU, the bacterial species *Streptococcus rubneri,* had a statistically significant Kruskal-Wallis *p*-value of 0.030 with an effect size of -1.5. However, after using the Benjamini–Hochberg procedure to control for FDR, the corrected *q*-value was 0.72, indicating no significance.

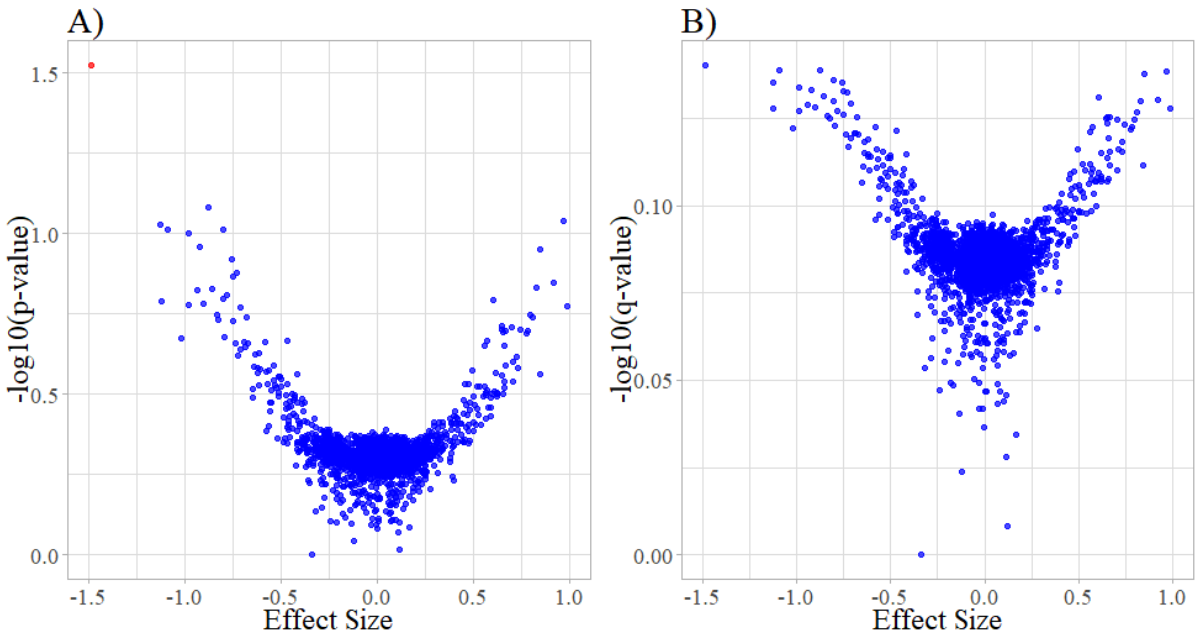


Figure 4.15 Volcano plot of the median standardised difference (effect size) between RT-PCR cases (n = 6) and their matched negative controls (n = 24). Colour indicates statistical significance level, with red representing a *p* or *q*-value < 0.05 and blue representing a *p* or *q*-value ≥ 0.05. A: Effect Size vs the - $\log_{10}$(Kruskal-Wallis *p*-value); B: Effect Size vs the - $\log_{10}$(Benjamini-Hochberg corrected *q*-value).

Figure 4.16 shows volcano plots of the effect size vs the Kruskal-Wallis *p*-value and Benjamini-Hochberg corrected *q*-value for those who met the symptom-based case definition vs those who did not. Overall, 56 OTUs showed a statistically significant negative change in effect size (decreased median differences) between those who met the definition vs those who did not using the uncorrected Kruskal-Wallis *p*-value. However, after using the Benjamini-Hochberg correction procedure, there was no significant difference found between the two groups.

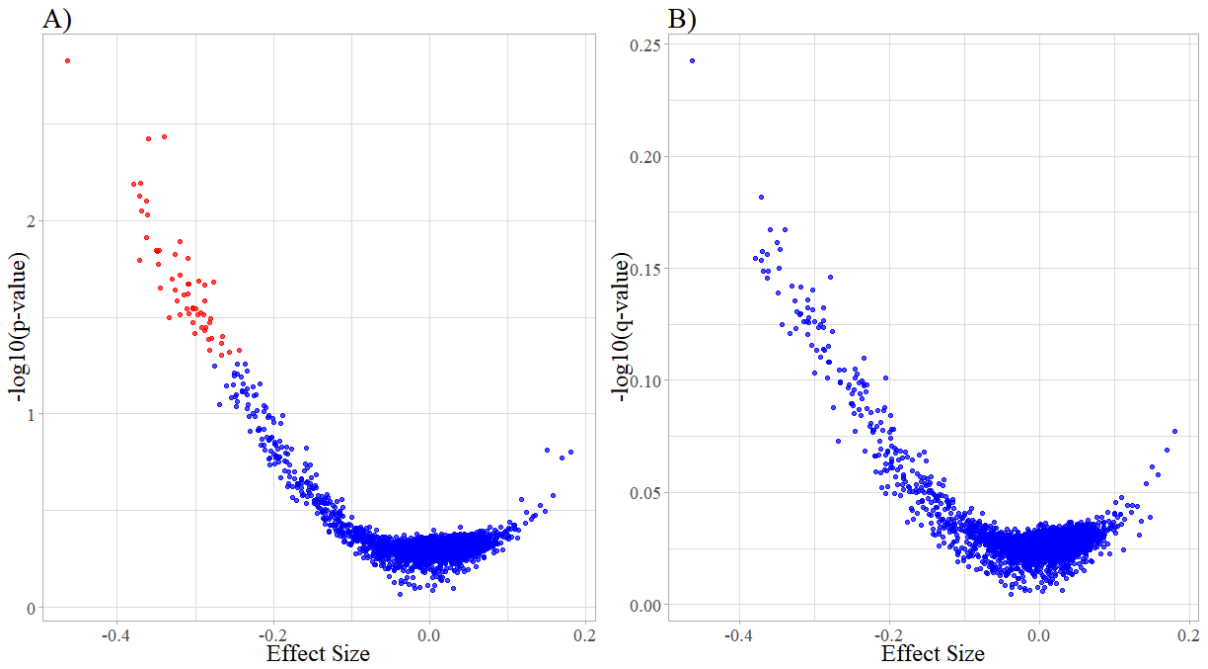


Figure 4.16 Volcano plot of the median standardised difference (effect size) between participants who met the symptom-based case definition (n = 37) vs those who did not (n = 79). Colour indicates statistical significance level, with red representing a *p* or *q*-value < 0.05 and blue representing a *p* or *q*-value ≥ 0.05. A: Effect Size vs the - $\log_{10}$(Kruskal-Wallis *p*-value); B: Effect Size vs the - $\log_{10}$(Benjamini-Hochberg corrected *q*-value).

Figure 4.17 shows volcano plots of the effect size vs the Kruskal-Wallis *p*-value and Benjamini-Hochberg corrected *q*-value for sequenced participants with positive vs negative serology. Overall, 17 OTUs showed a statistically significant change in effect size using the uncorrected Kruskal-Wallis *p*-value between those with positive vs negative serology, with 14 OTUs showing an increase in the median standardised difference and 3 OTUs a decrease. However, after the Benjamini-Hochberg correction procedure, no significant difference was found between those with positive vs negative serology.

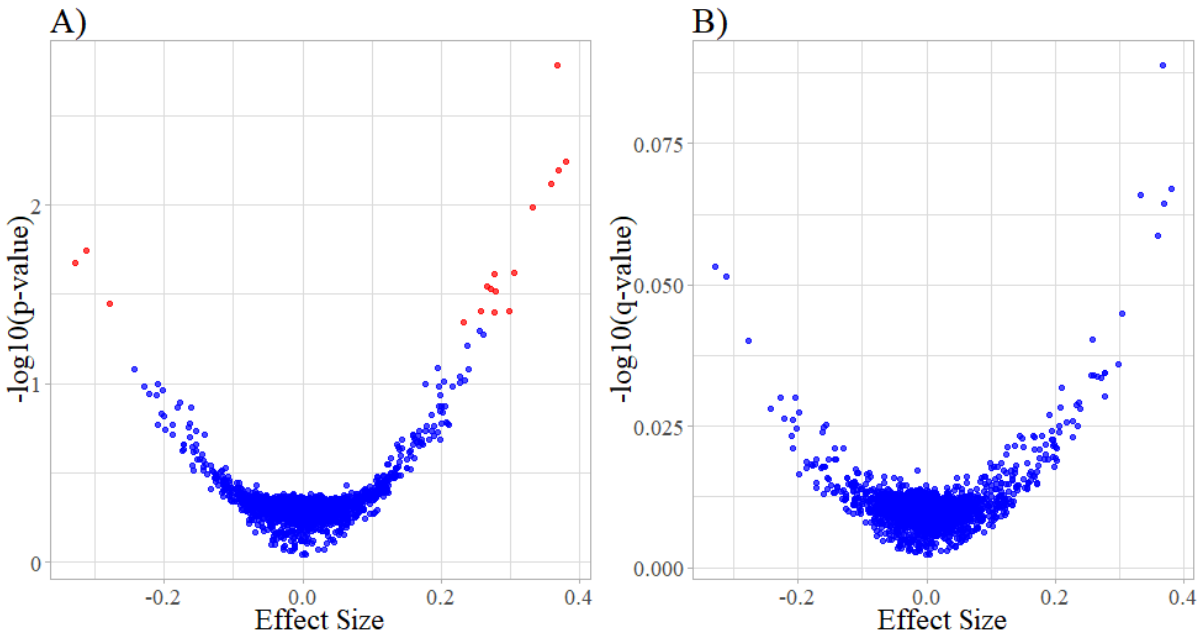


Figure 4.17 Volcano plot of the median standardised difference (effect size) between participants with positive serology (n = 68) vs negative serology (n = 43). Colour indicates statistical significance level, with red representing a *p* or *q*-value < 0.05 and blue representing a *p* or *q*-value ≥ 0.05. A: Effect Size vs the - $\log_{10}$(Kruskal-Wallis *p*-value); B: Effect Size vs the - $\log_{10}$(Benjamini-Hochberg corrected *q*-value).

Finally, Figure 4.18 shows volcano plots of the effect size vs the Kruskal-Wallis *p*-value and Benjamini-Hochberg corrected *q*-value for sequenced participants who had any evidence of current or previous SARS-CoV-2 infection vs those with no evidence. Six OTUs showed a statistically significant decrease in effect size using the uncorrected Kruskal-Wallis *p*-value between these two groups. However, after the Benjamini-Hochberg correction procedure, no significant difference was found between those with any evidence of current or previous SARS-CoV-2 infection vs sequenced participants with no evidence.

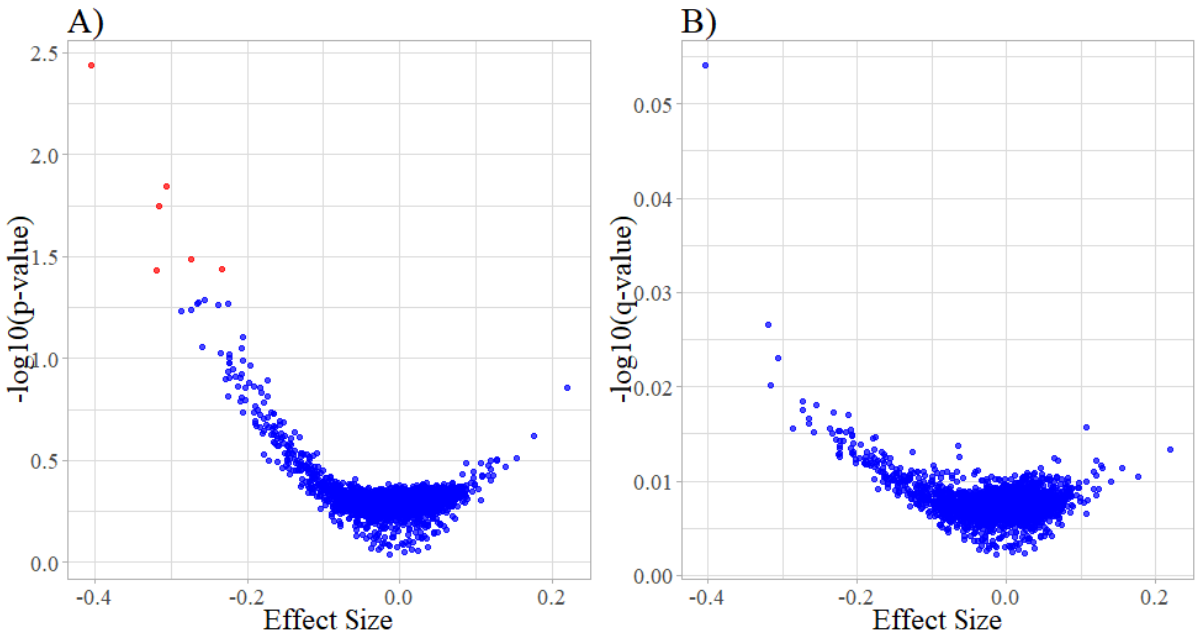


Figure 4.18 Volcano plot of the median standardised difference (effect size) between participants with any evidence of current or past SARS-CoV-2 infection (n = 80) vs no evidence (n = 36). Colour indicates statistical significance level, with red representing a *p* or *q*-value < 0.05 and blue representing a *p* or *q*-value ≥ 0.05. A: Effect Size vs the - $\log_{10}$(Kruskal-Wallis *p*-value); B: Effect Size vs the - $\log_{10}$(Benjamini-Hochberg corrected *q*-value); SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

4.4.4 Reported Health Behaviours

The reported health behaviours of participants who completed the survey were analysed across the different sampling time points. The proportion of responding participants who reported social distancing in each Round is shown in Figure 4.19. Social distancing behaviour did not change significantly between 20 April and 4 May 2020 (83.0% vs 79.4%, $p = 0.11$) but did decrease significantly between 4 May and 18 May 2020 (79.4% vs 65.7%, $p < 0.001$). Likewise, a further drop occurred between 18 May and 2 June 2020 (65.7% vs 48.1%, $p < 0.001$) and between 2 June and 17 June 2020 (48.1% vs 39.7%, $p = 0.012$). Lockdown restrictions underwent some changes between these dates. Changes which affected social distancing as defined in this study included: from 13 May 2020, people were allowed to meet one other person from outside their household outdoors; from 1 June 2020, rules allowed outdoor social gatherings of up to six people; and from 15 June 2020 non-essential retail shops reopened.[200]

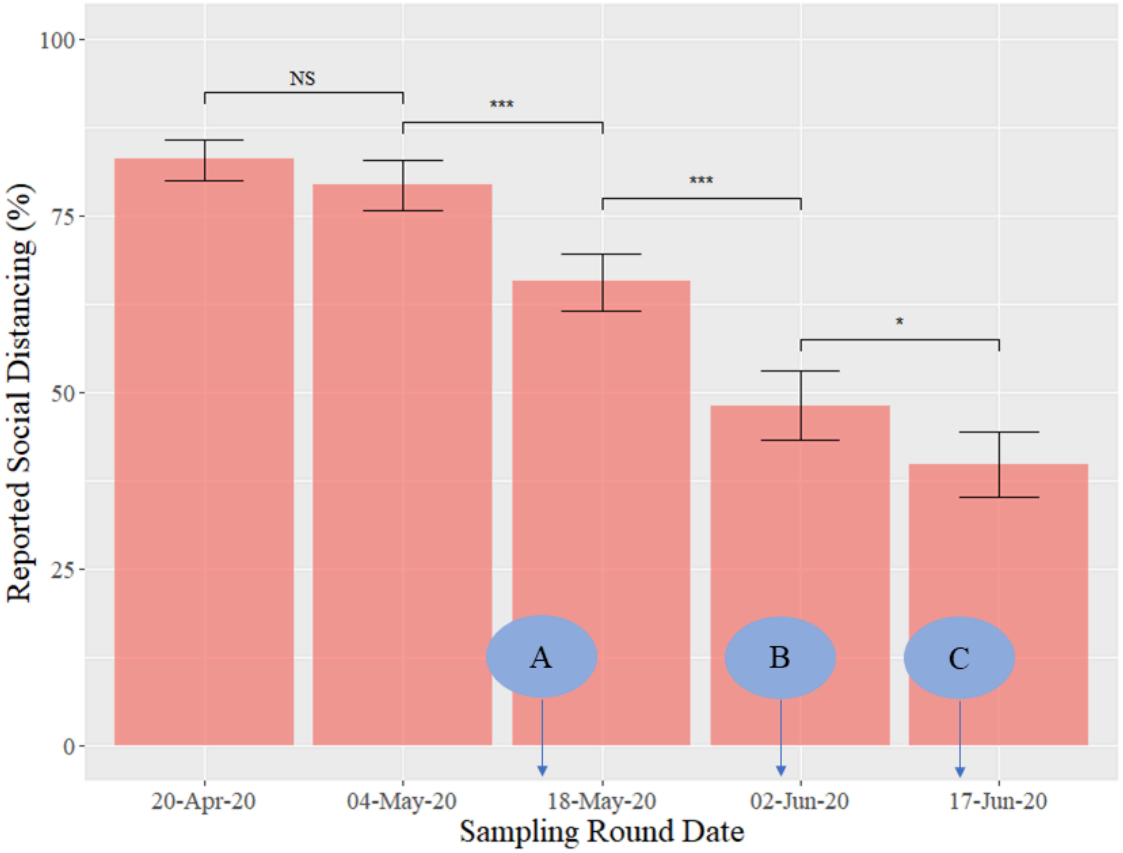


Figure 4.19 Reported social distancing behaviour by sampling Round date with 95% binomial confidence intervals. A two-sample and two-sided test for equality of proportions without Yates's correction for continuity was used to determine *p*-values with: not significant (NS), indicating $p > 0.05$, * $p < 0.05$ and *** $p < 0.001$. Changes to lockdown restrictions that occurred during the sampling period are indicated in blue circles with A: 13 May 2020, people allowed to meet one other non-household person outside; B: 1 June 2020 restrictions lifted on outdoor gatherings of up to six people; C: 15 June 2020 non-essential retail shops reopen.[200]

Multiple logistic regression was completed showing predictors for social distancing measures in the preceding seven days, as shown in Tables 4.17 and 4.18. The adjusted analysis included predictors that were not highly correlated, with results obtained after adjusting for sex, age, Round, household size, key worker status, occupation, education level, chronic medical conditions, current nicotine use and chronic respiratory allergies. Compared with Round 1, participants in Rounds 3, 4 and 5 were significantly less likely to report social distancing behaviour with adjusted ORs (95%CI) of 0.38 (0.29 - 0.49), 0.18 (0.14 - 0.24) and 0.13 (0.097 - 0.17) respectively with *p*-values < 0.001 for each.

Table 4.17 Multiple logistic regression on predictors for social distancing behaviour as a yes vs no dichotomous outcome variable. COVID-19: Coronavirus disease 2019; OR: Odds Ratio; CI: Confidence Interval.

	Unadjusted Analysis			Adjusted Analysis*		
Variable	β Coefficient	Unadjusted OR (95% CI)	*p*-value	β Coefficient	Adjusted OR (95% CI)	*p*-value
Sex						
Female	Referent	1	-	-	-	-
Male	0.085	1.1 (0.93 - 1.3)	0.30	0.051	1.1 (0.88 - 1.3)	0.57
Age						
Years	-0.00042	1.0 (1.0 - 1.0)	0.86	0.003	1.0 (1.0 - 1.0)	0.31
Round						
1	Referent	1	-	-	-	-
2	-0.23	0.79 (0.59 - 1.1)	0.11	-0.26	0.77 (0.58 - 1.0)	0.080
3	-0.94	0.39 (0.30 - 0.51)	<0.001	-0.97	0.38 (0.29 - 0.49)	<0.001
4	-1.7	0.19 (0.14 - 0.25)	<0.001	-1.7	0.18 (0.14 - 0.24)	<0.001
5	-2.0	0.14 (0.10 - 0.18)	<0.001	-2.0	0.13 (0.098 - 0.17)	<0.001
Household Size (number of occupants)						
1	Referent	1	-	-	-	-
2	0.42	1.5 (1.1 - 2.1)	0.011	0.18	1.2 (0.85 - 1.7)	0.29
3	0.34	1.4 (0.99 - 2.0)	0.054	0.16	1.2 (0.8 - 1.7)	0.42
4	0.27	1.3 (0.93 - 1.8)	0.12	0.067	1.1 (0.74 - 1.5)	0.72
5+	0.21	1.2 (0.87 - 1.8)	0.24	-0.068	0.94 (0.63 - 1.4)	0.73
Key Worker Status						
No/Unknown	Referent	1	-	-	-	-
Yes	-0.040	0.96 (0.78 - 1.2)	0.70	0.015	1.0 (0.78 - 1.3)	0.91
Main Economic Activity						
Paid Employment	Referent	1	-	-	-	-
Self-employed	-0.13	0.88 (0.67 - 1.2)	0.38	-	-	-
Student	0.12	1.1 (0.88 - 1.5)	0.35	-	-	-
Retired	-0.093	0.91 (0.72 - 1.2)	0.43	-	-	-
Other	0.0031	1 (0.71 - 1.4)	0.99	-	-	-
Occupation						
Professional	Referent	1	-	-	-	-
Health or social care	0.095	1.1 (0.81 - 1.5)	0.55	0.10	1.1 (0.76 - 1.6)	0.60
Business/administration	-0.27	0.76 (0.59 - 0.99)	0.042	-0.12	0.89 (0.66 - 1.2)	0.42
Other	-0.44	0.65 (0.49 - 0.86)	0.0028	-0.40	0.67 (0.48 - 0.93)	0.017
None	-0.073	0.93 (0.77 - 1.1)	0.46	-0.17	0.84 (0.68 - 1.1)	0.14
Highest level of education obtained						
Higher degree	Referent	1	-	-	-	-
Bachelor's or equivalent	-0.27	0.76 (0.64 - 0.91)	0.0032	-0.15	0.86 (0.70 - 1.1)	0.15
A-levels or equivalent	-0.41	0.67 (0.51 - 0.87)	0.0026	-0.036	0.96 (0.71 - 1.3)	0.82
O-levels or equivalent	-0.64	0.52 (0.35 - 0.79)	0.0016	-0.15	0.86 (0.55 - 1.4)	0.51
None	-0.12	0.88 (0.40 - 2.1)	0.77	0.41	1.5 (0.63 - 3.8)	0.37

***** Adjusted for sex, age, Round, household size, key worker status, occupation, education level, chronic medical conditions, current nicotine use and chronic respiratory allergies.

Those who reported working in "other" occupations, as defined in the methodology, were less likely to socially distance, with an adjusted OR (95% CI) of 0.67 (0.48 - 0.93) and a *p*-value

of 0.017 when compared to participants in professional occupations. As seen in Table 4.18, current nicotine users were less likely to socially distance, with the relationship strengthening after adjusting for other predictor variables, with an adjusted OR (95%CI) of 0.65 (0.47 - 0.90) and *p*-value of 0.0079.

Table 4.18 Multiple logistic regression on predictors for social distancing behaviour as a yes vs no dichotomous outcome variable. COVID-19: Coronavirus disease 2019; OR: Odds Ratio; CI: Confidence Interval.

	Unadjusted Analysis			Adjusted Analysis*		
Variable	β Coefficient	Unadjusted OR (95% CI)	*p*-value	β Coefficient	Adjusted OR (95% CI)	*p*-value
Chronic medical condition						
No	Referent	1	-	-	-	-
Yes	0.073	1.1 (0.89 - 1.3)	0.46	0.080	1.1 (0.87 - 1.3)	0.47
Current nicotine use						
No	Referent	1	-	-	-	-
Yes	-0.34	0.71 (0.54 - 0.94)	0.017	-0.43	0.65 (0.47 - 0.90)	0.0079
Chronic respiratory allergies						
No	Referent	1	-	-	-	-
Yes	0.14	1.2 (0.98 - 1.4)	0.082	0.10	1.1 (0.93 - 1.3)	0.26

* Adjusted for sex, age, Round, household size, key worker status, occupation, education level, chronic medical conditions, current nicotine use and chronic respiratory allergies.

Participants were also asked whether they were socially isolating on the day of sampling, with results by Round shown in Figure 4.20. Over time, the proportion of participants reporting social isolation decreased, but there was only one statistically significant drop between 4 and 18 May 2020 (7.0% vs 3.7%, $p = 0.016$). Out of the 74 participants who met the symptom-based case definition and were therefore required at the time to self-isolate at home, only 11 (14.9%) reported social isolation. Overall, 135 participants reported socially isolating in the five Rounds, with 19 (14.1%) isolating because of their symptoms, 11 (8.1%) because of a sick household contact, 5 (3.7%) because of an outside exposure to COVID-19 and the remaining 100 (74.1%) indicated they were isolating for "other" reasons not listed.

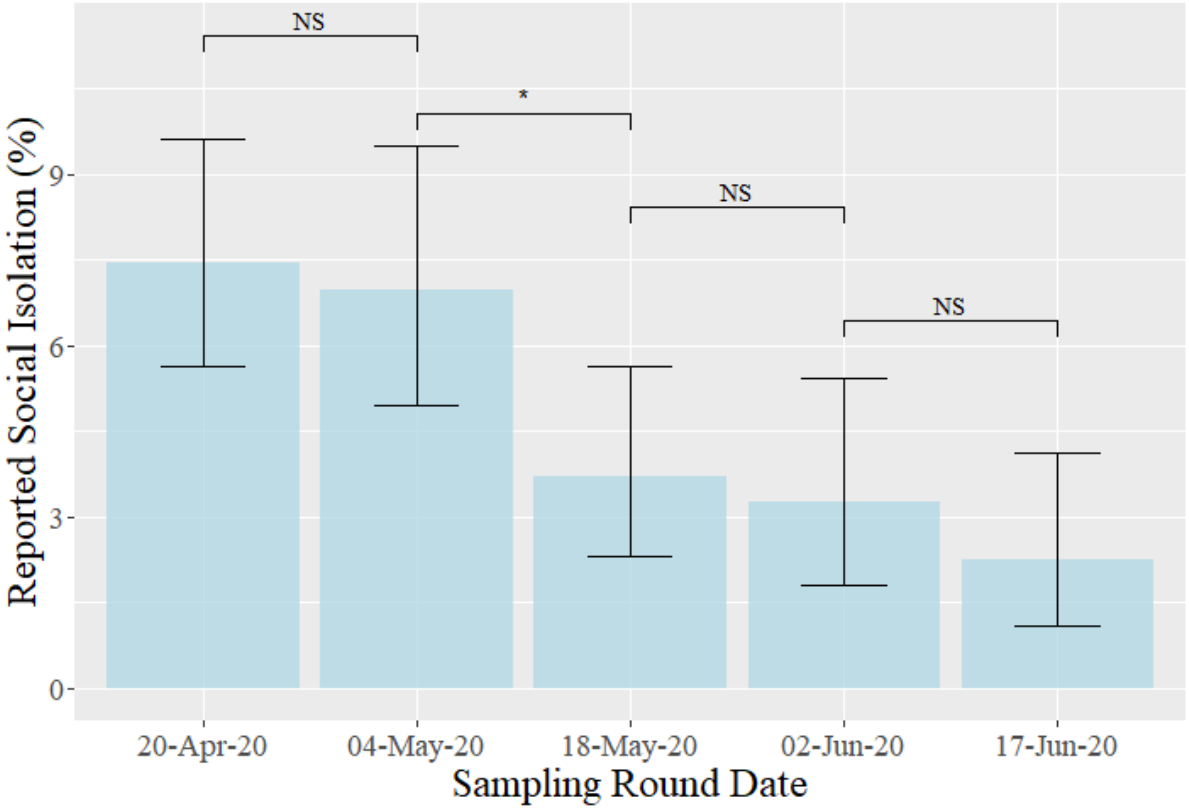


Figure 4.20 Reported social isolation on the day of testing by sampling Round date with 95% binomial confidence intervals. A two-sample and two-sided test for equality of proportions without Yates's correction for continuity was used to determine *p*-values with not significant (NS) indicating $p \geq 0.05$ and * $p < 0.05$.

All six RT-PCR positive participants reported adherence to lockdown rules on 20 April and 4 May 2020. However, none reported socially isolating at the time of sampling. As was seen in the univariate analysis in Table 4.8, participants who reported socially isolating at the time of testing were more likely to have evidence of current or previous SARS-CoV-2 infection with an unadjusted OR (95% CI) of 1.8 (1.1 - 3.0) and *p*-value of 0.023. However, social isolation was not a predictor of evidence for current or previous infection after adjusting for other factors with an adjusted OR (95% CI) of 1.6 (0.76 - 3.2) and *p*-value of 0.17.

Out of the 2,678 participants who completed the survey, 2,567 (95.9%) had left their property in the preceding week. These participants were asked about their handwashing habits and frequency of staying two-metres away from others while outside their property in the

preceding seven days. As shown in Figure 4.21, the proportion of participants who reported "Always" washing their hands on returning home decreased between Rounds. While there was no significant difference between sequential Rounds in the number of participants reporting "Always" washing their hands vs all remaining frequency categories, there was a significant drop from 75.5% in Round 1 to 67.9% in Round 4 (two-sided *p*-value of 0.0076).

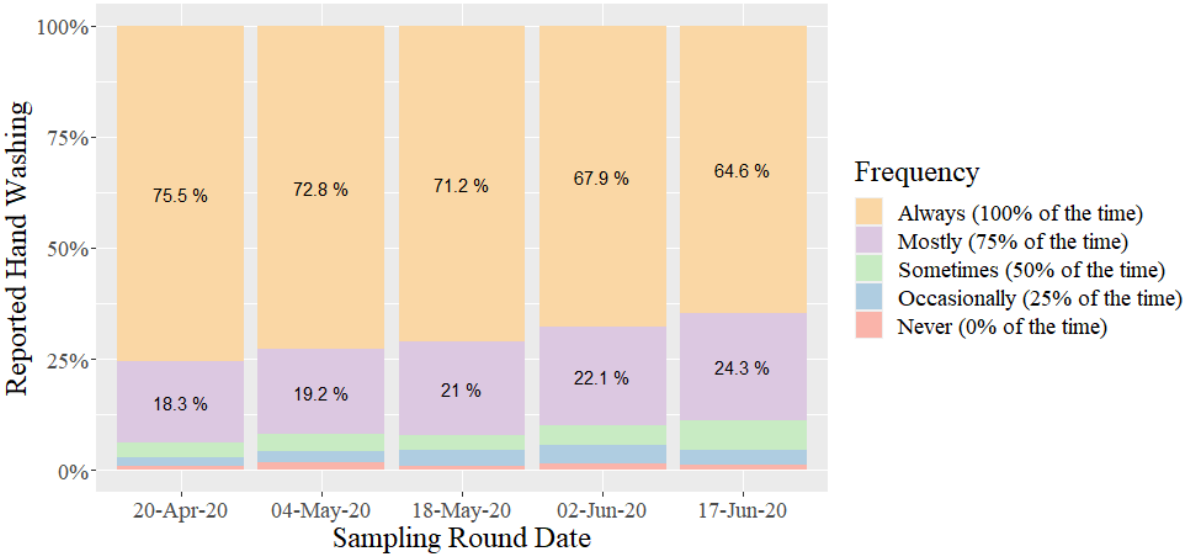


Figure 4.21 Reported participant hand washing frequency on returning home in the preceding seven days by Round for the subset of participants (n = 2,567) who reported leaving their property during this time period.

Similarly, the proportion of participants reporting "Always" staying a two-metre distance away from persons outside their household when outside their property dropped between Rounds, as shown in Figure 4.22. The proportion of participants reporting "Always" staying a two-metre distance from non-household members while outside their home compared with all remaining frequency categories decreased significantly from 49.3% to 38.1% between 20 April and 4 May 2020 (two-sided *p*-value 0.00015). The proportion of participants keeping a two-metre distance from others outside their property continued to decrease after 4 May 2020 but did not have a significant change between any other sequential sampling Round dates.

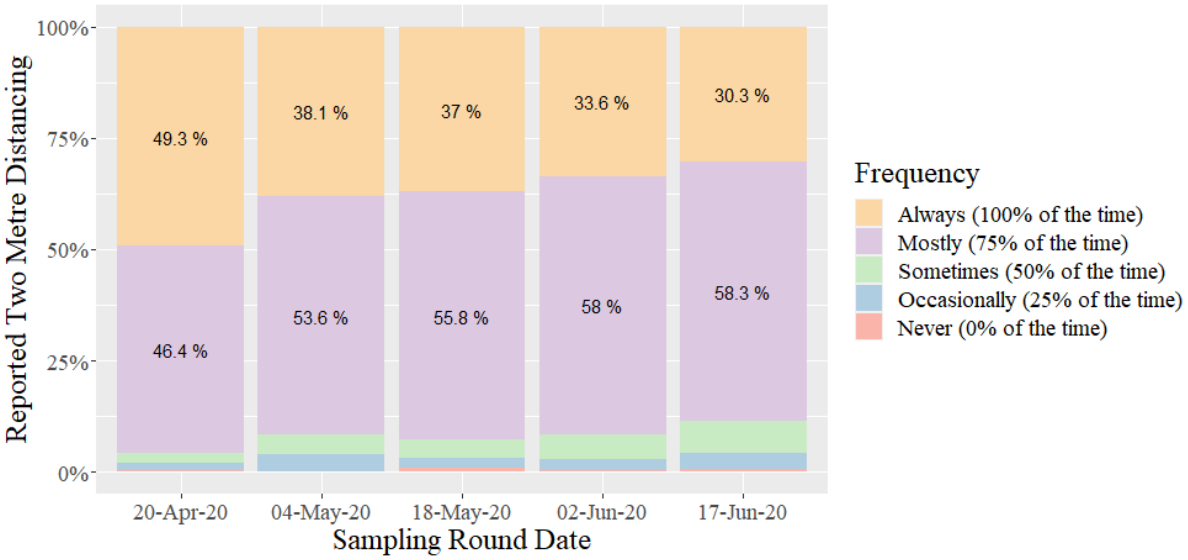


Figure 4.22 Reported two-metre distancing frequency while outside in the preceding seven days by Round for the subset of participants (n = 2,567) who reported leaving their property in this time period.

Section 4.5 Discussion

In this study, we collected samples from the adult population in Oxfordshire, UK to determine the community prevalence of SARS-CoV-2 infection between 20 April and 17 June 2020. We compared four diagnostic methods: RT-PCR, mNGS, serology and the symptom-based case definition and also collected data on other factors associated with transmission, such as information on individuals' social distancing behaviour.

We were able to determine the community prevalence of SARS-CoV-2 infection and monitor its change over time during the first few months of the COVID-19 pandemic in Oxfordshire. The estimate obtained on 20 April 2020 was earlier than any other community prevalence survey conducted in the UK. Subsequent community prevalence estimates from 4 May to 17 June 2020 in this study were comparable to ONS model estimates for England and the South East region and the REal-time Assessment of Community Transmission (REACT) surveys.[195,263] Combined with the evidence that the sample reasonably represented the area's

adult population, these results are likely accurate representations of SARS-CoV-2 infection prevalence during the study period in this sampled community.

While reliable findings, the community prevalence of SARS-CoV-2 between April - June 2020 was determined to be very low (<1%) and decreased over subsequent sampling Rounds. This result is likely due in part to the effectiveness of the first national UK lockdown enforced during the study. Commencing 23 March 2020 with stay-at-home orders, lockdown led to a rapid reduction in new SARS-CoV-2 infections in the UK within the first 14 days.[264] Prior to lockdown, modelled estimates of total UK SARS-CoV-2 infections based on critical care admissions suggested 527,000 (95% CrI: 362,000–797,000) cumulative cases had already occurred by 23 March 2020 with a doubling time of 2.8 days (95% CrI: 2.6–3.0 days) at that time.[199] Only a week prior, a few days after a pandemic was declared by the WHO and this study designed, the infection doubling time in the UK was estimated to be 3.8 days.[228] While the true community prevalence of SARS-CoV-2 infection in Oxfordshire was unknown before 20 April 2020, it was likely much higher in the weeks preceding sampling.

In this study, participants with positive SARS-CoV-2 RT-PCR results did not significantly differ from those with negative results across various characteristics, including sex, age group, occupation and education level. However, RT-PCR positive participants were significantly more likely to meet the symptom-based case definition and have positive serology (*p*-values 0.011 and 0.0052, respectively). When compared to RT-PCR as the gold standard, the symptom-based case definition and serology tests both had a very low PPV and high FDR, reflective of the low SARS-CoV-2 infection prevalence in the sample population. The overall accuracy (95% CI) of the symptom-based case definition 97.1% (96.4 - 97.7%)

was significantly higher than serology 93.2% (92.1 - 94.3%) when compared using RT-PCR results at the observed prevalence level.

In addition, the prevalence-independent DORs for both the symptom-based case definition and serology tests were high (17.7 and 14.1, respectively), with the former outperforming the latter. The positive likelihood ratios for both the symptom-based case definition and serology tests indicated that a positive result significantly increased the post-test probability of truly having COVID-19. However, their negative likelihood ratios suggested that a negative result was not significantly associated with having or not having COVID-19 when using RT-PCR as the gold standard.

This result may be due to features of the disease course of SARS-CoV-2 infection illustrated in Figure 4.1. In a cross-sectional study design, a "snapshot" is taken of each participant on the day of testing, but it is unknown where in the disease course they may be at the time. RT-PCR positivity can occur in the pre-symptomatic phase and before antibody production occurs at detectable levels. In addition, other pathogens can cause SARS-CoV-2 symptoms, and serology positivity can persist beyond the RT-PCR positive window. Asymptomatic infections likely constitute a substantial portion of the cases.[211] In this current study, the majority of SARS-CoV-2 RT-PCR positive participants had no symptoms, suggesting they were either asymptomatic or pre-symptomatic. In such instances, the symptom-based case definition is rendered less or completely ineffective.

Despite this, the sensitivity and specificity for the symptom-based case definition found in this study (33.3% and 97.3%, respectively) were higher than in other literature. For example, one study based in Tamale found a sensitivity of 17.5% and specificity of 88.1% respectively

for fever and cough, with a sensitivity and specificity of 24.7% and 89.7% respectively for loss of taste or smell.[265] Similarly, serology tests in this study had a sensitivity and specificity (50.0% and 93.4%) for SARS-CoV-2 infection higher than in previous research. One study using VivaDiag® to screen symptomatic patients for current COVID-19 infection had a sensitivity of 30% and specificity of 89% compared with RT-PCR.[266] Overall, the low number of true positives as determined by RT-PCR means that there cannot be high confidence in the sensitivity results of any of the alternative diagnostic methods, as demonstrated by their large confidence intervals. However, the specificity results are likely more reliable given the large sample size.

Nanopore mNGS results were not significantly associated with RT-PCR positivity for SARS-CoV-2 infection, with a *p*-value 0.094. This may be due to the low prevalence of the disease in the population and low sample size leading to a type II error, where the null hypothesis fails to be rejected. Given the large proportions of unclassified reads and the use of self-collected oropharyngeal swabs, the sample quality may have also been low. This study was conducted before the widespread UK community use of self-collected nasopharyngeal and oro-nasopharyngeal swabs, which had previously been viewed as unreliable because of participant discomfort and the assumed need for formal training.[267] Nasopharyngeal swabs were later found to be superior to oropharyngeal swabs in diagnosing COVID-19, with self-collection in the community sensitive on testing and acceptable to participants.[268–270]

Another contributor to the insignificant mNGS findings could be the high C_T values in the majority of the RT-PCR positive participants in this study. High C_T values are associated with lower viral loads, which makes mNGS detection less likely. For example, it has been found that for RT-PCR C_T values < 30 the sensitivity of nanopore mNGS for detection of

SARS-CoV-2 is 100% (95%CI: 84.5 - 100%), while high C_T values ≥ 30 have a sensitivity of 50% (95%CI: 27.8 - 72.0%).[236] This finding tracks with this current study, where both participants with C_T values < 30 were positive on mNGS, while none with C_T > 30 were positive. In a study which used samples with low C_T values < 25, improved taxonomic binning tools were able to sensitively and specifically detect SARS-CoV-2, even in the absence of reference sequences.[61] This work highlights the potential of mNGS to detect known and unknown pathogens accurately, but indicates that a higher viral load may be necessary.

The findings of the logistic regression on any evidence of current or previous infection vs no evidence can mostly be explained by the low PPV and high FDR of mNGS, serology and the symptom-based case definition in this study due to the low population prevalence. It would be expected that no predictors for SARS-CoV-2 infection would be found if the majority of the cases with 'evidence' for SARS-CoV-2 infection were spurious false positive results. The exception to this is the finding in Round 5, where there may have been a true association between lower evidence of SARS-CoV-2 infection when compared with Round 1, given the more rural geographical location for this Round. It has since been found that early in the pandemic, those living in rural locations in England had a lower infection risk than those in urban areas, which supports this conclusion.[271]

The microbiome analysis did not show any significant difference in alpha diversity, beta diversity or differential abundance for the four comparison groups examined (RT-PCR positive cases vs negative controls, those that met the symptom-based case definition vs those who did not, positive vs negative serology and any evidence of current or previous SARS-CoV-2 infection vs no evidence). This failure to reject H_0 may be because of no true

difference in microbiome diversity measurements, or because of the low PPV and high FDR found for each different SARS-CoV-2 detection method when compared against the gold standard of RT-PCR. Finding no diversity alterations between these groups would be consistent, as it would not be expected that participants with false positive test results would differ significantly in their oropharyngeal microbiome from those with true negative results if false positive results occur at random.

For the microbiome in the SARS-CoV-2 RT-PCR positive group vs negative controls, the failure to reject H_0 across diversity metrics may be due to the underpowered sample size, given that the oropharyngeal community composition is known to undergo changes during SARS-CoV-2 infection.[218] However, before this current study microbiome analyses for COVID-19 have not been conducted in the wider population, with most studies being limited to symptomatic cases in hospitals. It is possible that the result showing no diversity differences is related to the majority of the RT-PCR positive participants being asymptomatic at the time of their test. The microbiome of the nasopharynx is known to have greater changes in beta diversity in those with more severe disease symptoms.[219] Therefore, on the opposite end of the spectrum, asymptomatic cases may truly have no detectable microbiome alterations. It is possible that symptom appearance is related to microbiome changes, perhaps in the context of the host immune response. This could warrant future investigation given the complex interplay between SARS-CoV-2 infection and the host microbiome.

While there were too few SARS-CoV-2 RT-PCR positive participants to subdivide the microbiome analysis into symptomatic and asymptomatic cases in this current study, there appeared to be a greater relative abundance of *Haemophilus parainfluenzae* in participants with positive SARS-CoV-2 RT-PCR results. While not statistically significant, this finding

would be consistent with a previous study.[272] It is possible that a larger sample size with division into symptomatic and asymptomatic cases would have detected a significantly greater relative abundance of *Haemophilus parainfluenzae*.

For the health behaviours examined, there was a significant decrease in self-reported social distancing after 4 May 2020. This may be due to the easing of some lockdown restrictions that occurred during the study period outside of the scope of the survey's ability to capture. For example, people could meet one other non-household person outside their household outdoors from 13 May 2020, and this was not covered in the survey as the questionnaire was set before the rules changed.[200] However, the reduction in social distancing could have also been due to other factors, as previously found in subsequent comparable compliance studies.[224,225] Interestingly, age was not a predictor of social distancing behaviour, nor was having a chronic medical condition, despite the COVID-19 disease risk being greater in these populations. This may have been due to individuals in these categories receiving at-home care assistance, a nuance that the survey questions were not designed to capture.

In the multiple logistic regression, participants who currently smoked or used e-cigarettes were significantly less likely to report social distancing even after controlling for other variables, with an adjusted OR (95%CI) of 0.65 (0.47 - 0.90) and *p*-value of 0.0079. This could be due to differences in perceived risks and benefits of behavioural compliance, given that smoking is associated with increased time preference with higher delay discounting.[273] Delay discounting occurs when immediate, smaller rewards are preferred over larger, delayed rewards. In this case, social distancing guidelines required forgoing immediate rewards such as visiting loved ones for delayed benefits, including reducing COVID-19 transmission and the end of the restrictions on human movement. In support of this hypothesis, it has been

found that adherence to social distancing is inversely associated with delay discounting.[274] Nonetheless, current smokers have been found to have reduced odds of SARS-CoV-2 infection, suggesting a complex interaction that could be investigated in future research.[275]

In this study, only 14.9% of participants who reported symptoms which met the case definition for COVID-19 also reported socially isolating. This result is lower than comparable findings in the literature, such as one paper which found a 24.9% rate of social isolation compliance when cough or fever symptoms are present.[276] In addition, health behaviours such as hand washing and keeping a two-metre distance from others consistently dropped between Rounds despite public health messaging on these measures not changing during the study period. It has been found that compliance measurements can vary depending on how public health guidance messages are conveyed, but this study had the strength of using the same survey questions across time points, eliminating this potential bias.[277] It is possible the decline was due to moral disengagement increasing over the period of the first lockdown, as has been theorised in one study.[278]

4.5.1 Strengths and Limitations

A large strength of this study was its ability to be conducted in the early stages of the COVID-19 pandemic in the UK. This research benefited from a large number of participants with a good response rate across a range of metrics, including survey completion, serology test results reported, and the number of swabs returned. However, response rates did consistently decrease subsequently between each Round. This decline was potentially due to unusually high volunteer motivation at the start of the pandemic waning over subsequent weeks and months.

While this study relied upon a volunteer sampling method, which is known to introduce bias, the number of applications received meant that stratified randomised sampling could be conducted to select participants using sex and age group categories in line with 2011 UK census data. Ultimately, it is difficult to mitigate volunteer and consent bias when conducting research in human populations using ethical principles, and it is possible the sample population differed in significant ways from non-recruited Oxfordshire residents. For example, reported rates of paid employment, key worker status and household size were comparable to population estimates.[238,256] However, participants in this study had other characteristics that differed, such as higher educational attainment than 2011 census data for the Oxfordshire population.[257]

A major limitation of this study was the sample size, given the low community prevalence of SARS-CoV-2 infection during the study period. This led to only a small number of SARS-CoV-2 infections detectable by RT-PCR. When this study was designed in March 2020, the sample size calculation could not take into account the effects of lockdown that would later be announced and enforced. The ONS COVID-19 Infection Survey later found community prevalence levels in England as high as 7.6% (95% CI 7.4 -7.8%) in March - April 2022.[229] Therefore, it was not unreasonable at the time to assume that a 4% prevalence, which was used for the sample size calculation, could be reached. Instead, prevalence dropped as low as 0.03% (95%CI 0.01 - 0.06%) on 17 June 2020 in the South East region of England.[258] At this prevalence level, the sample size required to compare different SARS-CoV-2 detection methods would have been 320,000 participants, greater than 100 times the size of the original study. This highlights the difficulty in conducting research comparing novel pathogen detection methods when the true prevalence of a disease is very low.

Because of the rarity of SARS-CoV-2 infection in the sample population, there were limitations to the types of statistical tests that could be performed and many of the results had wide confidence intervals. Overall, the study was broadly underpowered to detect associations between SARS-CoV-2 infection as measured by RT-PCR positivity and some of the independent variables of interest. A strength of this study is that the statistical tests chosen were appropriate for this low count data; however, the low number of true positives increased the probability of type II errors.

4.5.2 Conclusion and Future Directions

This Chapter provided a serial cross-sectional estimate of SARS-CoV-2 infection prevalence in a representative Oxfordshire population between April - June 2020, with community sampling starting, to the best of our knowledge, at an earlier time point than anywhere else in the UK. Three alternative detection methods were compared against RT-PCR as the gold standard, however, the low number of true positives limited the interpretability of alternative test sensitivity. Future studies conducted in higher prevalence settings could be used to compare different detection approaches. While this study found no changes in alpha or beta diversity metrics in SARS-CoV-2 cases, the majority of cases were asymptomatic at the time of testing and future work could investigate whether microbiome alterations only occur in the presence of symptoms. Given the declining rates of social distancing, hand washing and keeping a two-metre distance from others over time found in this study, future studies could model whether the reduction in preventive health behaviours affects the effectiveness of prolonged lockdowns in reducing disease transmission.

4.5.3 Data Availability

The de-identified data used in this Chapter is available on request in compliance with ethical requirements for transfer and sharing of participant-collected data, along with all code used to generate the results, tables and figures.

5

Discussion

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Section 5.1 Summary

This thesis has explored detection for novel pathogens with pandemic potential from different angles. It is the first, to the best of our knowledge, to systematically analyse current global considerations of novel diseases in national public health infrastructure. We have offered insights into how the different detection thresholds for new diseases used by countries around the world may impact two important public health metrics: the time-to-detection and size at outbreak discovery. We investigated random testing in the population, determined the level of surveillance coverage required to detect SARS-CoV-2 outbreaks before the first case

presentation to hospital under model assumptions, and explored how this varies for disease outbreaks with different transmission dynamics and public health surveillance targets. Finally, we examined a range of diagnostic approaches in the early stages of the COVID-19 pandemic and determined early serial estimates of SARS-CoV-2 infection prevalence in a representative adult community population in Oxfordshire, United Kingdom (UK). Our findings have highlighted the importance of early detection and the potential of random population sampling to uncover asymptomatic and mild cases in the initial stages of a pandemic. Our research can be summarised broadly into *current detection* for novel diseases and its implications, and *future detection* using different strategies and diagnostic approaches.

5.1.1 Current Detection

We began in Chapter Two by examining the current surveillance infrastructure for novel diseases in 195 signatory countries to the International Health Regulations (2005). Our research systematically compiled public resources to search for evidence of novel disease considerations in national public health laws, notifiable disease lists, health professional guidelines, and laboratory guidelines. Our results demonstrated that only a minority of the countries examined had evidence of the inclusion of novel diseases in their national public health surveillance infrastructure. Consideration of novel diseases in public health surveillance differed by World Health Organisation (WHO) region, with evidence of inclusion significantly less likely in Africa and the Eastern Mediterranean when compared with the European region. Countries with high income and health expenditure were more likely to have considered novel diseases in their public health surveillance. This finding reiterates calls for increased investment in public health and providing assistance to bolster surveillance in under-resourced settings.[279,280]

We noted that formal novel disease consideration is not the only mechanism used to detect new pathogen outbreaks. Event-based surveillance (EBS) can also potentially detect epidemic events in a hypothesis-free fashion even if new diseases are not explicitly considered, serving as another mechanism for novel pathogen outbreak detection.[281] However, the Global Health Security Index in 2021 has highlighted that only a minority of countries operate EBS systems.[48] In Chapter Two and Appendix C, we found novel disease consideration in countries was highly correlated with having EBS, indicating low global coverage of novel disease surveillance either through explicit consideration as determined by our research, or implicit inclusion through EBS.

In Chapter Three, we examined the implications of different detection thresholds found for reporting novel disease outbreaks between different countries which consider novel diseases in surveillance. We determined that for outbreaks with SARS-CoV-2 transmission dynamics, countries which do not report new diseases until the first case presentation to a health service can have significant detection delays and a greater outbreak size at discovery. Our results demonstrated that almost all SARS-CoV-2 outbreaks which reach at least one case in hospital will continue to grow to significantly larger sizes without going extinct on their own. By the time there are four hospitalised cases, as was the situation when SARS-CoV-2 was first recognised in Wuhan, China, more than two months (64 days) may have elapsed since the first case exposure and the true outbreak size is predicted to be greater than 300 times larger than the number of known cases. Our findings were in agreement with other estimates in the literature, increasing the confidence in our model results.[142] For diseases with a lower symptom or hospital presentation probability than SARS-CoV-2, there can be even greater detection delays and larger outbreak size at discovery using current surveillance.

In Chapter Four, we presented a study conducted in the early stages of the Coronavirus Disease (COVID-19) pandemic. At the time, the community prevalence of SARS-CoV-2 infection was unknown in the UK, and it was particularly unclear given the uncertain level of mild or asymptomatic cases. This study was implemented in the context of low availability and access to diagnostic testing, and we showcased some of the difficulties faced in detection when prevalence is very low and a substantial proportion of the cases are asymptomatic. Our prevalence results were consistent with later findings from the Office for National Statistics (ONS) and REal-time Assessment of Community Transmission (REACT) surveys.[195,263] We demonstrated that the symptom-based case definition and serology tests were significantly associated with RT-PCR positivity and had high diagnostic odds ratios. However, we noted that such detection methods would not be available in the earliest stages of a novel pathogen outbreak. One method that would be available is metagenomic next-generation sequencing (mNGS) given its ability to be used in a hypothesis-free pathogen-agnostic fashion.[62,282] While mNGS did not demonstrate a significant association with SARS-CoV-2 RT-PCR positivity and infection-induced changes in the oropharyngeal microbiomes in our research, this was likely in part attributable to our small sample size. Given that other studies have demonstrated a strong association, the potential of mNGS for pathogen-agnostic surveillance should not be dismissed and warrants further investigation.[160,236]

Overall, our research has highlighted a current global deficiency in infrastructure to detect novel diseases. Early detection of a new pathogen outbreak is an incredibly demanding public health objective, given it requires surveillance in a previously disease-free population and discovery at low prevalence levels.[165] However, we have shown that delayed discovery using current surveillance methods can lead to large outbreak sizes upon detection. Increased outbreak sizes at discovery can have a significant impact on the ability of the public health

response to reduce transmission and contain a new disease given the growth in resources required.[283] Detection of asymptomatic and pre-symptomatic cases are important components of outbreak control, and our research has shown this is possible though population-level sampling even at very low levels of disease prevalence.[147]

5.1.2 Future Detection

In addition to our investigation of current surveillance for new diseases, our research has highlighted some of the potential directions forward to improve detection for novel pathogen outbreaks with pandemic potential. In Chapter Two, we underscored the benefits of standardising novel disease detection and advocated for the explicit inclusion of novel diseases in public health surveillance. In particular, public health laws are a useful tool for pandemic policy implementation and could be expanded to include provisions for novel disease detection.[127] There is a need for standardising approaches to novel pathogen surveillance, as lack of guidance impedes reporting of cases to public health authorities.[106] Our findings highlighted a low level of novel disease consideration in laboratory guidelines in particular, despite their importance for pandemic preparedness.[284] Currently, only a minority of countries have access to laboratories that can perform the identification of unknown pathogens, but expansion of access is possible.[111] Future public health policy could incorporate explicit guidelines on novel pathogen discovery in laboratory settings, and arrange for sample sharing with established international partners in low-resource settings.

In Chapter Three, we examined how random sampling in the population could lead to earlier outbreak discovery compared with current detection in hospitals or within a set target number of days. This could be possible through pathogen-agnostic testing, such as mNGS in clinical samples. We demonstrated that for certain parameters, such as the probability of symptoms or

length of the latent period, the surveillance coverage level required to detect an outbreak differs depending on whether the discovery target set by public health authorities is within the a set number of days or prior to the first hospital presentation, the latter representing traditional detection. Our results showed that for diseases with higher symptom probability than SARS-CoV-2, random sampling may be infeasible given the high level of coverage required to detect an outbreak before traditional surveillance may detect the new disease in a healthcare setting. On the other hand, novel diseases that are characterised by substantial asymptomatic or pre-symptomatic transmission may only necessitate minimal population surveillance coverage to detect an outbreak before traditional surveillance could achieve this through healthcare presentations. Even with a small amount of sampling, random population surveillance via mNGS could be invaluable in the event of another novel pathogen emergence similar to HIV-1, as discussed in Chapter One, given the potentially years-long asymptomatic transmission phase characteristic of some viruses.

We discussed in Chapter Three the potential for wastewater detection as an alternative to random population sampling using clinical samples. Since the start of the COVID-19 pandemic, there has been an expansion of research on the use of wastewater epidemiology for viral outbreak detection.[188,189] There have been calls to use mNGS on wastewater samples to improve surveillance for novel disease threats.[285] Given the potential pathogen-agnostic benefits, future research could seek to determine whether detection of novel outbreaks through wastewater is feasible and if this approach is sensitive enough to outperform traditional surveillance relying upon symptomatic healthcare presentations.

In Chapter Four, we demonstrated that random sampling in a community population was able to detect SARS-CoV-2 infection, even at a very low prevalence level. We highlighted some of

the potential of mNGS to detect SARS-CoV-2 infection, although we interpret our results with caution given the small sample sizes. Our results demonstrated that in cases with at least an intermediate viral load, as determined by C_T values on RT-PCR testing, mNGS is able to reliably identify the presence of the SARS-CoV-2 genome in clinical samples. This is consistent with other research findings.[236] In the presence of a low viral load, mNGS detection capabilities are more limited. If mNGS is used for novel pathogen detection in a future surveillance system, this limitation will need to be accounted for or addressed through innovations in mNGS technology and laboratory protocols.

Overall, our work highlights various improvements that could be made to improve future detection for novel pathogen outbreaks. Some changes could be easier to implement than others. For example, countries could work now to ensure novel disease considerations in public health laws, notifiable disease lists, health professional guidelines and laboratory guidelines are at least at parity around the world. The potentially forthcoming Pandemic Treaty could be used to help ensure all nations are resourced to take steps to detect future novel disease outbreaks.[128] This could include universalising access in lower-resourced countries to laboratories with the ability to identify unknown pathogens, given only a minority have this capability or existing international collaborations for this purpose.[111] Implementing pathogen-agnostic surveillance using mNGS technology has been called for previously and our research has highlighted some of the benefits in terms of improved time-to-detection and outbreak size at discovery.[67,160] This would be significantly more challenging and feasibility assessments would need to consider the required coverage levels our research has uncovered in addition to other factors. It is possible wastewater detection or other forms of aggregate sampling using mNGS may overcome some of the barriers of individual-based testing and could be considered for future surveillance system designs.

Section 5.2 Future Research Directions

The research presented in this thesis emphasises the critical role of early detection and highlights some of the existing gaps in novel disease surveillance. To achieve robust public health preparedness worldwide, it is imperative for the global community to enhance its ability to detect new pathogen outbreaks before the next pandemic threat occurs. Advancing this goal necessitates ongoing research and development in surveillance, novel disease detection standards and laboratory science. The findings presented in this thesis could be expanded upon through follow-up studies investigating a range of important directions we have uncovered through our work. Our suggested future research proposals are summarised in Table 5.1, along with the intended audience for the research outputs and their potential benefits.

Table 5.1 Summary of suggested future research directions to improve detection for novel pathogen pandemics. mNGS: metagenomic next-generation sequencing.

Category	Suggested Future Research	Audience	Potential Benefits
Knowledge Building	Use Appendix B to compare all features of national public health surveillance systems, including reporting practices, timeliness and nationally notifiable disease list inclusions	Public Health Practitioners	Global assessment of surveillance system performance; uncover areas for improvement
	Compile non-public novel disease surveillance resources and practices from public health departments worldwide	Policymakers; Public Health Practitioners	Improve the understanding of current surveillance capabilities; track progress; share best practice
Standards Development	Use Appendix B and further research with expert input to collate and create protocols and standards for laboratory detection of novel pathogens	Public Health Laboratories; Public Health Practitioners	Establish and share a best practice gold standard for laboratories; accelerate early detection and pathogen characterisation
	Use Appendix B and further research to collate and create guidelines and a standard for health practitioner reporting of suspected novel diseases	Doctors; Community Health Workers; Public Health Practitioners	Establish and share a best practice gold standard for novel disease reporting; improve early detection
	Conduct a public health law study to compare national approaches, monitor and assess novel disease inclusion, and develop effective legal standards	Lawmakers; Academics; Policymakers	Determine the best legal instruments for novel disease surveillance and pandemic preparedness; share best practice between countries
Surveillance System	Periodic global survey study to monitor whether novel diseases are on nationally notifiable disease lists	Policymakers; Public Health Practitioners	Track and incentivise explicit inclusion of novel diseases in public health surveillance
	Conduct a modelling study of random population surveillance incorporating test sensitivity and sampling frequency	Public Health Practitioners; Academics	Develop insights into optimal future surveillance system design
	Use network modelling to identify pivotal geographical points of disease spread	Policymakers; Academics	Prioritise hotspot locations for optimal early warning system placement
	Cost-effectiveness studies of population surveillance for novel diseases using i) clinical sampling and ii) wastewater	Public Health Practitioners; Academics	Understand the economic implications; determine optimal surveillance strategy
	Model optimal integration of different event-based surveillance datastreams with random population sampling	Public Health Practitioners; Academics	Determine cost-effective surveillance strategy; improve early detection
Laboratory Science	Conduct case-control studies to assess mNGS detection of various pathogens without library reference sequences	Academics; Public Health Laboratories	Further examine mNGS pathogen-agnostic capabilities to detect future novel diseases; determine potential range of mNGS sensitivity

Building on the results in Chapter Two, future research could attempt to access and compile non-public domain resources from public health departments worldwide. This would lead to a richer understanding of current surveillance infrastructure and could be used to track updates and progress. In addition, the large dataset we compiled (Appendix B) for our research could be used to create a standard from existing resources for novel pathogen surveillance. The availability of detection standards would be particularly valuable for health professionals faced with uncertainty in the diagnosis and reporting of new diseases. It would also be of great utility in laboratory preparedness if protocols for how to manage a clinical sample of a suspected novel disease were available.

Models incorporating pathogen-agnostic approaches to surveillance could be expanded upon beyond our results in Chapter Three, trialling real-world pilot studies of population-based surveillance using mNGS technology. Incorporation of test sensitivity would be an essential consideration of future research given its importance in early detection systems.[165] Researchers could explore new approaches that integrate EBS, such as using an aberration signal from wearable devices to guide targeted mNGS screening in clinical or wastewater settings. This could increase the chance of novel disease detection through targeted sequencing and decrease the time between epidemic commencement and pathogen characterisation, while potentially lowering costs through reducing sampling requirements. Our work did not consider implementation costs and follow-up work could investigate a cost-benefit analysis of surveillance inclusive of pathogen-agnostic mechanisms such as mNGS. Future research incorporating network modelling could assist in identifying major network nodes such as international transport hubs that could be prioritised for stronger novel pathogen detection capabilities.

Some of our work in Chapter Four was limited by the low prevalence of SARS-CoV-2 infection in our study population. Future research could conduct a case-control study to determine the utility of mNGS in hypothesis-free situations to see how it can detect other unknown disease targets. This has been shown to be possible for SARS-CoV-2, with the detection of a novel coronavirus even without reference sequences available.[236] Further research on different viral species and families using similar techniques could allow for better predictions on how this technology might perform in the case of the next Disease X outbreak.

Section 5.3 Conclusion

There are various obstacles in the detection of currently unknown infectious disease threats, and the early stages of an outbreak are often clouded by epidemiological uncertainty. Louis Pasteur emphasised this challenge in 1878 when he stated, "All things are hidden, obscure and debatable if the cause of the phenomena is unknown, but everything is clear if its cause be known."[286] Despite the challenges, reducing the time from obscurity to clarity early in an outbreak may be possible through the implementation of pathogen-agnostic approaches in disease surveillance. This is especially important in the context of currently unknown diseases, and in situations where there could be substantial numbers of asymptomatic cases.

Pathogen-agnostic surveillance, if it incorporates technologies such as mNGS, could also provide the tools necessary to rapidly proceed from the detection of the outbreak threat to the identification of the pathogen itself. Our ability to detect the next Disease X is growing, and we should harness new laboratory capabilities to the best of our ability. There are still various obstacles in terms of technological integration and public health infrastructure overhauls that would need to be overcome to arrive at the new form of global infectious disease surveillance

argued for in 2018.[67] However, the benefits of early detection and pathogen identification make pursuing further research in this area worthwhile. Detecting future novel pathogen outbreaks that are like SARS-CoV-2 or HIV-1 weeks, months, or even years before current surveillance methods permit could potentially save a large number of lives and livelihoods and prevent pandemics that threaten to become global catastrophic events.

Bibliography

1. Virchow R. Gesammelte Abhandlungen aus dem Gebiete der öffentlichen Medicin und der Seuchenlehre [Collected treatises from the field of public medicine and epidemic]. Vol. 1. Berlin, Germany: A. Hirschwald; 1879.

2. Samarasekera U. Investigating the origins of novel pathogens. Lancet Infect Dis. 2021 Dec 1;21(12):1633.

3. Fan VY, Jamison DT, Summers LH. Pandemic risk: how large are the expected losses? Bull World Health Organ. 2018 Feb 1;96(2):129–34.

4. Chiu CY. Viral pathogen discovery. Curr Opin Microbiol. 2013 Aug;16(4):468–78.

5. Chatterjee P, Nair P, Chersich M, Terefe Y, Chauhan AS, Quesada F, et al. One Health, "Disease X" & the challenge of "Unknown" Unknowns. Indian J Med Res. 2021 Mar;153(3):264–71.

6. Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis. 1995 Jan-Mar;1(1):7–15.

7. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008 Feb 21;451(7181):990–3.

8. Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci. 2001 Jul 29;356(1411):983–9.

9. Carlson CJ, Albery GF, Merow C, Trisos CH, Zipfel CM, Eskew EA, et al. Climate change increases cross-species viral transmission risk. Nature. 2022 Jul;607(7919):555–62.

10. Glennon EE, Bruijning M, Lessler J, Miller IF, Rice BL, Thompson RN, et al. Challenges in modeling the emergence of novel pathogens. Epidemics. 2021 Dec;37:100516.

11. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature. 2004 Jul 8;430(6996):242–9.

12. Woolhouse M, Gaunt E. Ecological origins of novel human pathogens. Crit Rev Microbiol. 2007;33(4):231–42.

13. Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, VandePol S. Rapid evolution of RNA genomes. Science. 1982 Mar 26;215(4540):1577–85.

14. Steinhauer DA, Holland JJ. Rapid evolution of RNA viruses. Annu Rev Microbiol. 1987;41:409–33.

15. Hay AJ, Gregory V, Douglas AR, Lin YP. The evolution of human influenza viruses. Philos Trans R Soc Lond B Biol Sci. 2001 Dec 29;356(1416):1861–70.

16. MacIntyre CR. Biopreparedness in the Age of Genetically Engineered Pathogens and Open Access Science: An Urgent Need for a Paradigm Shift. Mil Med. 2015 Sep;180(9):943–9.

17. Lipsitch M, Inglesby TV. Moratorium on research intended to create novel potential pandemic pathogens. MBio. 2014 Dec 12;5(6).

18. Manheim D, Lewis G. High-risk human-caused pathogen exposure events from 1975-2016. F1000Res. 2021 Aug 4;10:752.

19. West RM, Gronvall GK. CRISPR Cautions: Biosecurity Implications of Gene Editing. Perspect Biol Med. 2020;63(1):73–92.

20. Bond J. CRISPR and COVID-19: Lessons Learned to Prepare for the Next Pathogen. Georgetown Scientific Research Journal. 2022 Feb 17;2(1):24-41.

21. O'Brien JT, Nelson C. Assessing the Risks Posed by the Convergence of Artificial Intelligence and Biotechnology. Health Security. 2020 May/Jun;18(3):219–27.

22. Inglesby TV, Relman DA. How likely is it that biological agents will be used deliberately to cause widespread harm? Policymakers and scientists need to take seriously the possibility that potential pandemic pathogens will be misused. EMBO Rep. 2016 Feb;17(2):127–30.

23. Sandberg A, Nelson C. Who Should We Fear More: Biohackers, Disgruntled Postdocs, or Bad Governments? A Simple Risk Chain Model of Biorisk. Health Security. 2020 Jun 10;18(3):155–63.

24. Barras V, Greub G. History of biological warfare and bioterrorism. Clin Microbiol Infect. 2014 Jun;20(6):497–502.

25. Carus WS. The history of biological weapons use: What we know and what we don't. Health Secur. 2015 Jul;13(4):219–55.

26. Malet D. The Pursuit and Use of Biological Weapons by States. Oxford Research Encyclopedia of International Studies.

27. Gronvall GK. Synthetic Biology: Biosecurity and Biosafety Implications. In: Singh SK, Kuhn JH, editors. Defense Against Biological Attacks: Volume I. Cham: Springer International Publishing; 2019. p. 225–32.

28. DiEuliis D, Ellington AD, Gronvall GK, Imperiale MJ. Does Biotechnology Pose New Catastrophic Risks? Curr Top Microbiol Immunol. 2019;424:107–19.

29. Schoch-Spana M, Cicero A, Adalja A, Gronvall G, Kirk Sell T, Meyer D, et al. Global Catastrophic Biological Risks: Toward a Working Definition. Health Secur. 2017 Jul 26;15(4):323–8.

30. Yassif JM, Korol S, Kane A. Guarding Against Catastrophic Biological Risks: Preventing State Biological Weapon Development and Use by Shaping Intentions. Health Secur. 2023 Apr 26.

31. Thompson RN, Gilligan CA, Cunniffe NJ. Will an outbreak exceed available resources

for control? Estimating the risk from invading pathogens using practical definitions of a severe epidemic. Journal of the Royal Society Interface. 2020 Nov 25;17(172):13.

32. Diekmann O, Heesterbeek JAP, Metz JAJ. On the definition and the computation of the basic reproduction ratio R0 in models for infectious diseases in heterogeneous populations. J Math Biol. 1990 Jun 1;28(4):365–82.

33. Singer BJ, Thompson RN, Bonsall MB. The effect of the definition of "pandemic" on quantitative assessments of infectious disease outbreak risk. Sci Rep. 2021 Jan 28;11(1):2547.

34. Chan EH, Brewer TF, Madoff LC, Pollack MP, Sonricker AL, Keller M, et al. Global capacity for emerging infectious disease detection. Proc Natl Acad Sci U S A. 2010 Dec 14;107(50):21701–6.

35. Perkins MD, Dye C, Balasegaram M, Bréchot C, Mombouli JV, Røttingen JA, et al. Diagnostic preparedness for infectious disease outbreaks. Lancet. 2017 Nov 11;390(10108):2211–4.

36. Guest C, Ricciardi W, Kawachi I, Lang I. Oxford Handbook of Public Health Practice. OUP Oxford; 2013 Feb 28.

37. Johnson GA, Vindrola-Padros C. Lessons for Current and Future Pandemics. In: Vindrola-Padros C, Johnson GA, editors. Caring on the Frontline during COVID-19: Contributions from Rapid Qualitative Research. Singapore: Springer Singapore; 2022. p. 293–305.

38. Thompson RN, Thompson CP, Pelerman O, Gupta S, Obolski U. Increased frequency of travel in the presence of cross-immunity may act to decrease the chance of a global pandemic. Philos Trans R Soc Lond B Biol Sci. 2019 Jun 24;374(1775):20180274.

39. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. Antiviral Res. 2018 Nov;159:63–7.

40. Simpson S, Kaufmann MC, Glozman V, Chakrabarti A. Disease X: accelerating the development of medical countermeasures for the next pandemic. Lancet Infect Dis. 2020 May;20(5):e108–15.

41. Simpson S, Chakrabarti A, Robinson D, Chirgwin K, Lumpkin M. Navigating facilitated regulatory pathways during a disease X pandemic. NPJ Vaccines. 2020 Oct 23;5:101.

42. Steele L, Orefuwa E, Dickmann P. Drivers of earlier infectious disease outbreak detection: a systematic literature review. Int J Infect Dis. 2016 Dec;53:15–20.

43. World Health Organization. International Health Regulations (2005) Third Edition. 3rd ed. Geneva: World Health Organization; 2016. 1-84 p.

44. Balajee SA, Salyer SJ, Greene-Cramer B, Sadek M, Mounts AW. The practice of event-based surveillance: concept and methods. Global Security: Health, Science and Policy. 2021 Jan 1;6(1):1–9.

45. Bellizzi S, Cegolon L, Bubbico L, Ferlito S, Farina G, Pichierri G. The importance of event-based surveillance for preparedness and response in future respiratory pandemics. J Glob Health. 2021 Aug 21;11:03098.

46. Shakeri Hossein Abad Z, Kline A, Sultana M, Noaeen M, Nurmambetova E, Lucini F, et al. Digital public health surveillance: a systematic scoping review. NPJ Digit Med. 2021 Mar 3;4(1):41.

47. Kuehne A, Keating P, Polonsky J, Haskew C, Schenkel K, De Waroux OLP, et al. Event-based surveillance at health facility and community level in low-income and middle-income countries: a systematic review. BMJ global health. 2019;4(6):e001878.

48. Bell JA, Nuzzo JB. Global Health Security Index: Advancing Collective Action and Accountability Amid Global Crisis, 2021 [Internet]. Nuclear Threat Initiative and Johns Hopkins Center for Health Security; 2021 Dec 8 [cited 2022 Jul 1].

49. Ganser I, Thiébaut R, Buckeridge DL. Global Variations in Event-Based Surveillance for Disease Outbreak Detection: Time Series Analysis. JMIR Public Health Surveill. 2022 Oct 31;8(10):e36211.

50. Edelstein M, Lee LM, Herten-Crabb A, Heymann DL, Harper DR. Strengthening Global Public Health Surveillance through Data and Benefit Sharing. Emerg Infect Dis. 2018 Jul;24(7):1324.

51. Bagherian H, Farahbakhsh M, Rabiei R, Moghaddasi H, Asadi F. National Communicable Disease Surveillance System: A review on Information and Organizational Structures in Developed Countries. Acta Inform Med. 2017 Dec;25(4):271–6.

52. Husain M, Rahman M, Alamgir A, Uzzaman MS, Flora MS. Disease surveillance system of Bangladesh: combating public health emergencies. Online J Public Health Inform. 2019;11(1).

53. Murray J, Cohen AL. Infectious Disease Surveillance. International Encyclopedia of Public Health. 2016 Oct 24;222-9.

54. Meadows AJ, Oppenheim B, Guerrero J, Ash B, Badker R, Lam CK, et al. Infectious Disease Underreporting Is Predicted by Country-Level Preparedness, Politics, and Pathogen Severity. Health Security. 2022 Aug 1;20(4):331–8.

55. McGowan CR, Takahashi E, Romig L, Bertram K, Kadir A, Cummings R, Cardinal LJ. Community-based surveillance of infectious diseases: a systematic review of drivers of success. BMJ Global Health. 2022 Aug 1;7(8):e009934.

56. Rainer TH, Cameron PA, Smit D, Ong KL, Hung ANW, Nin DCP, et al. Evaluation of WHO criteria for identifying patients with severe acute respiratory syndrome out of hospital: prospective observational study. BMJ. 2003 Jun 21;326(7403):1354–8.

57. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: challenges and pitfalls. Virus Res. 2014 Dec 19;194:175–83.

58. Zhu X, Yan S, Yuan F, Wan S. The Applications of Nanopore Sequencing Technology in Pathogenic Microorganism Detection. Can J Infect Dis Med Microbiol. 2020 Dec 31;2020:6675206.

59. Ai JW, Zhang Y, Zhang HC, Xu T, Zhang WH. Era of molecular diagnosis for pathogen identification of unexplained pneumonia, lessons to be learned. Emerg Microbes Infect. 2020 Mar 16;9(1):597–600.

60. Wu X, Li Y, Zhang M, Li M, Zhang R, Lu X, et al. Etiology of Severe Community-Acquired Pneumonia in Adults Based on Metagenomic Next-Generation Sequencing: A Prospective Multicenter Study. Infect Dis Ther. 2020 Dec;9(4):1003–15.

61. Chandrakumar I, Gauthier NPG, Nelson C, Bonsall MB, Locher K, Charles M, et al. BugSplit enables genome-resolved metagenomics through highly accurate taxonomic binning of metagenomic assemblies. Commun Biol. 2022 Feb 22;5(1):151.

62. Gauthier NPG, Chorlton SD, Krajden M, Manges AR. Agnostic sequencing for detection of viral pathogens. Clin Microbiol Rev. 2023 Feb 27;e0011922.

63. Miller RR, Montoya V, Gardy JL, Patrick DM, Tang P. Metagenomics for pathogen detection in public health. Genome Med. 2013 Sep 20;5(9):81.

64. Govender KN, Street TL, Sanderson ND, Eyre DW. Metagenomic Sequencing as a Pathogen-Agnostic Clinical Diagnostic Tool for Infectious Diseases: a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies. J Clin Microbiol. 2021 Aug 18;59(9):e0291620.

65. Mohsin H, Asif A, Fatima M, Rehman Y. Potential role of viral metagenomics as a surveillance tool for the early detection of emerging novel pathogens. Arch Microbiol. 2021 Apr;203(3):865–72.

66. Bassi C, Guerriero P, Pierantoni M, Callegari E, Sabbioni S. Novel Virus Identification through Metagenomics: A Systematic Review. Life. 2022 Dec 7;12(12).

67. Gardy JL, Loman NJ. Towards a genomics-informed, real-time, global pathogen surveillance system. Nat Rev Genet. 2018 Jan;19(1):9–20.

68. Bohl JA, Lay S, Chea S, Ahyong V, Parker DM, Gallagher S, et al. Discovering disease-causing pathogens in resource-scarce Southeast Asia using a global metagenomic pathogen monitoring system. Proceedings of the National Academy of Sciences. 2022;119(11):e2115285119.

69. Reid M, Enanoria W, Stoltey J, Philip S, Fuchs J, Lockwood A, et al. The SARS-CoV-2 pandemic: the race to trace: contact tracing scale-up in San Francisco—early lessons learned. J Public Health Policy. 2021 Jun 1;42(2):211–21.

70. Kretzschmar ME, Rozhnova G, van Boven M. Isolation and Contact Tracing Can Tip the Scale to Containment of COVID-19 in Populations With Social Distancing. Frontiers in Physics. 2021 Feb;8:677.

71. Robinson ER, Walker TM, Pallen MJ. Genomics and outbreak investigation: from

sequence to consequence. Genome Med. 2013 Apr 29;5(4):36.

72. Paulik LB, Keenan RE, Durda JL. The Case for Effective Risk Communication: Lessons from a Global Pandemic. Integr Environ Assess Manag. 2020 Sep;16(5):552–4.

73. Covello VT, McCallum DB, Pavlova MT. Effective Risk Communication: The Role and Responsibility of Government and Nongovernment Organizations. Springer Science & Business Media; 2012.

74. Kluberg SA, Mekaru SR, McIver DJ, Madoff LC, Crawley AW, Smolinski MS, et al. Global Capacity for Emerging Infectious Disease Detection, 1996-2014. Emerg Infect Dis. 2016 Oct;22(10):E1–6.

75. Team WS, Others. Ebola haemorrhagic fever in Sudan, 1976. Bull World Health Organ. 1978;56(2):247–70.

76. Feldmann H, Jones S, Klenk HD, Schnittler HJ. Ebola virus: from discovery to vaccine. Nat Rev Immunol. 2003 Aug;3(8):677–85.

77. Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ. 1978;56(2):271-93.

78. Breman JG, Heymann DL, Lloyd G, McCormick JB, Miatudila M, Murphy FA, et al. Discovery and Description of Ebola Zaire Virus in 1976 and Relevance to the West African Epidemic During 2013–2016. J Infect Dis. 2016 Oct 4;214(S3):S93–101.

79. Morbidity and Mortality Weekly Report. Pneumocystis pneumonia-Los Angeles. Centers for Disease Control and Prevention; 1981;30:250-2.

80. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983 May 20;220(4599):868–71.

81. Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM, Ho DD. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. Nature. 1998 Feb 5;391(6667):594–7.

82. Pandey A, Galvani AP. The global burden of HIV and prospects for control. Lancet HIV. 2019 Dec;6(12):e809–11.

83. Morbidity and Mortality Weekly Report. Outbreak of Hendra-Like Virus -- Malaysia and Singapore, 1998-1999. Centers for Disease Control and Prevention; 1999 Apr;48(13);265-269.

84. Chua KB. Nipah virus outbreak in Malaysia. J Clin Virol. 2003 Apr;26(3):265–75.

85. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet. 1999 Oct 9;354(9186):1257–9.

86. Ambat AS, Zubair SM, Prasad N, Pundir P, Rajwar E, Patil DS, et al. Nipah virus: A review on epidemiological characteristics and outbreaks to inform public health decision making. J Infect Public Health. 2019 Feb 23;12(5):634–9.

87. Looi LM, Chua KB. Lessons from the Nipah virus outbreak in Malaysia. Malays J Pathol. 2007 Dec;29(2):63–7.

88. Xu RH, He JF, Evans MR, Peng GW, Field HE, Yu DW, et al. Epidemiologic clues to SARS origin in China. Emerg Infect Dis. 2004 Jun;10(6):1030–7.

89. de Benoist AC, Boccia D. WHO initiates enhanced global surveillance in response to acute respiratory syndrome in southern China, Vietnam, and Hong Kong, special administrative region (SAR) of China. Weekly releases (1997–2007). Eurosurveillance. 2003 Mar 13;7(11):2177.

90. Lee JW, McKibbin WJ. Globalization and disease: The case of SARS. Asian Econ Pap. 2004 Jan;3(1):113–31.

91. Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. The Lancet. 2015 Sep 5;386(9997):995-1007.

92. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012 Nov 8;367(19):1814–20.

93. Butler D. Tensions linger over discovery of coronavirus. Nature. 2013 Jan 14;10.

94. Zaki AM. Novel coronavirus - Saudi Arabia: human isolate [Internet]. ProMED-mail. 2012 Sep 20 [cited 2022 May 4].

95. Memish ZA, Perlman S, Van Kerkhove MD, Zumla A. Middle East respiratory syndrome. Lancet. 2020 Mar 28;395(10229):1063–77.

96. International Society for Infectious Diseases] PRO/AH/EDR> Undiagnosed pneumonia - China (HU): RFI [Internet]. ProMED-mail. 2019 Dec 30 [cited 2023 Mar 8].

97. The-nCoV Outbreak Joint Field Epidemiology Investigation Team, Li Q. An Outbreak of NCIP (2019-nCoV) Infection in China - Wuhan, Hubei Province, 2019-2020. China CDC Weekly. 2020 Jan 31;2(5):79–80.

98. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497–506.

99. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Feb 20;382(8):727–33.

100. Bauch CT, Oraby T. Assessing the pandemic potential of MERS-CoV. Lancet. 2013 Aug 24;382(9893):662–4.

101. Naser AM, Hossain MJ, Sazzad HMS, Homaira N, Gurley ES, Podder G, et al. Integrated cluster- and case-based surveillance for detecting stage III zoonotic pathogens: an example of Nipah virus surveillance in Bangladesh. Epidemiol Infect. 2015 Jul;143(9):1922–30.

102. Anderson RM, Fraser C, Ghani AC, Donnelly CA, Riley S, Ferguson NM, et al. Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic.

Philos Trans R Soc Lond B Biol Sci. 2004 Jul 29;359(1447):1091–105.

103. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med. 2020 Mar 13;27(2).

104. Davies S. Pandemics and the consequences of COVID‐19. Economic Affairs. 2020 Jun;40(2):131.

105. World Health Organization. IHR Core Capacity Monitoring Framework: Checklist and Indicators for Monitoring Progress in the Development of IHR Core Capacities in States Parties. Geneva:World Health Organization; 2013. Report No. WHO/HSE/GCR/2013.

106. MacDonald E, Aavitsland P, Bitar D, Borgen K. Detection of events of public health importance under the international health regulations: a toolkit to improve reporting of unusual events by frontline healthcare workers. BMC Public Health. 2011 Sep 21;11:713.

107. Drosten C, Preiser W, Günther S, Schmitz H, Doerr HW. Severe acute respiratory syndrome: identification of the etiological agent. Trends Mol Med. 2003 Aug;9(8):325–7.

108. Coleman CM, Frieman MB. Emergence of the Middle East respiratory syndrome coronavirus. PLoS Pathog. 2013 Sep 5;9(9):e1003595.

109. Koopmans M. Surveillance strategy for early detection of unusual infectious disease events. Curr Opin Virol. 2013 Apr;3(2):185–91.

110. Fernandez C, Beeching NJ. Pyrexia of unknown origin. Clin Med. 2018 Mar;18(2):170–4.

111. World Health Organisation. Electronic State Parties Self-Assessment Annual Reporting Tool [Internet]. 2023 [cited 2023 Jan 19].

112. Hartley DM, Nelson NP, Walters R, Arthur R, Yangarber R, Madoff L, et al. Landscape of international event-based biosurveillance. Emerg Health Threats J. 2010;7.

113. Vlieg WL, Fanoy EB, van Asten L, Liu X, Yang J, Pilot E, et al. Comparing national infectious disease surveillance systems: China and the Netherlands. BMC Public Health. 2017 Dec;17(1):415.

114. Turner AM, Liddy ED, Bradley J, Wheatley JA. Modeling public health interventions for improved access to the gray literature. J Med Libr Assoc. 2005 Oct;93(4):487–94.

115. Franks H, Hardiker NR, McGrath M, McQuarrie C. Public health interventions and behaviour change: reviewing the grey literature. Public Health. 2012 Jan;126(1):12–7.

116. Adams J, Hillier-Brown FC, Moore HJ, Lake AA, Araujo-Soares V, White M, et al. Searching and synthesising "grey literature" and "grey information" in public health: critical reflections on three case studies. Syst Rev. 2016 Sep 29;5(1):164.

117. Morss JR. The international legal status of the Vatican/Holy See complex. Eur J Int Law.

2015;26(4):927–46.

118. Rethlefsen ML, Kirtley S, Waffenschmidt S, Ayala AP, Moher D, Page MJ, et al. PRISMA-S: an extension to the PRISMA statement for reporting literature searches in systematic reviews. J Med Libr Assoc. 2021 Apr 1;109(2):174–200.

119. World Bank. World Bank Country and Lending Groups [Internet]. The World Bank Group. 2023 [cited 2023 Feb 15].

120. World Bank. Current health expenditure per capita [Internet]. The World Bank Group. 2022 [cited 2023 Feb 15].

121. Torres Munguía JA, Badarau FC, Díaz Pavez LR, Martínez-Zarzoso I, Wacker KM. A global dataset of pandemic- and epidemic-prone disease outbreaks. Sci Data. 2022 Nov 10;9(1):683.

122. R Core Team. R: A language and environment for statistical computing [Internet]. R Foundation for Statistical Computing, Vienna, Austria; 2022.

123. Islam SRU, Akther T, Sultana S, Deb P, Ghosh AK, Nasif MAO, et al. Challenges in the establishment of a biosafety testing laboratory for COVID-19 in Bangladesh. J Infect Dev Ctries. 2021 Dec 31;15(12):1833–7.

124. de Sousa R, Reusken C, Koopmans M. MERS coronavirus: data gaps for laboratory preparedness. J Clin Virol. 2014 Jan;59(1):4–11.

125. Marais G, Hardie D, Brink A. A case for investment in clinical metagenomics in low-income and middle-income countries. Lancet Microbe. 2023 Mar 1;4(3):e192-9.

126. Allen T, Murray KA, Zambrana-Torrelio C, Morse SS, Rondinini C, Di Marco M, et al. Global hotspots and correlates of emerging zoonotic diseases. Nat Commun. 2017 Oct 24;8(1):1124.

127. Martin R, Conseil A, Longstaff A, Kodo J, Siegert J, Duguet AM, et al. Pandemic influenza control in Europe and the constraints resulting from incoherent public health laws. BMC Public Health. 2010 Sep 3;10:532.

128. Hannon E, Hanbali L, Lehtimaki S, Schwalbe N. Why we still need a pandemic treaty. Lancet Glob Health. 2022 Sep;10(9):e1232–3.

129. Phelan AL, Carlson CJ. A treaty to break the pandemic cycle. Science. 2022 Jul 29;377(6605):475–7.

130. Carpenter TE, O'Brien JM, Hagerman AD, McCarl BA. Epidemic and economic impacts of delayed detection of foot-and-mouth disease: a case study of a simulated outbreak in California. J Vet Diagn Invest. 2011 Jan;23(1):26–33.

131. Ferguson NM, Donnelly CA, Anderson RM. Transmission intensity and impact of control policies on the foot and mouth epidemic in Great Britain. Nature. 2001 Oct 4;413(6855):542–8.

132. Lipsitch M, Cohen T, Cooper B, Robins JM, Ma S, James L, et al. Transmission dynamics and control of severe acute respiratory syndrome. Science. 2003 Jun

20;300(5627):1966–70.

133. Buckeridge DL, Burkom H, Campbell M, Hogan WR, Moore AW. Algorithms for rapid outbreak detection: a research synthesis. J Biomed Inform. 2005 Apr 1;38(2):99–113.

134. Buckeridge DL. Outbreak detection through automated surveillance: A review of the determinants of detection. J Biomed Inform. 2007 Aug 1;40(4):370–9.

135. Zhou H, Burkom H, Winston CA, Dey A, Ajani U. Practical comparison of aberration detection algorithms for biosurveillance systems. J Biomed Inform. 2015 Oct 1;57:446–55.

136. Xing J, Burkom H, Tokars J. Method selection and adaptation for distributed monitoring of infectious diseases for syndromic surveillance. J Biomed Inform. 2011 Dec 1;44(6):1093–101.

137. Parnell S, Gottwald TR, Gilks WR, van den Bosch F. Estimating the incidence of an epidemic when it is first discovered and the design of early detection monitoring. J Theor Biol. 2012 Jul 21;305:30–6.

138. Metz JA, Wedel M, Angulo AF. Discovering an epidemic before it has reached a certain level of prevalence. Biometrics. 1983 Sep;39(3):765–70.

139. Parnell S, Gottwald TR, Cunniffe NJ, Alonso Chavez V, van den Bosch F. Early detection surveillance for an emerging plant pathogen: a rule of thumb to predict prevalence at first discovery. Proceedings of the Royal Society B: Biological Sciences. 2015 Sep 7;282(1814):20151478.

140. Roberts DL, Rossman JS, Jarić I. Dating first cases of COVID-19. PLoS Pathog. 2021 Jun 24;17(6):e1009620.

141. Pekar J, Worobey M, Moshiri N, Scheffler K, Wertheim JO. Timing the SARS-CoV-2 index case in Hubei province. Science. 2021 Apr 23;372(6540):412–7.

142. Imai N, Dorigatti I, Cori A, Riley S, Ferguson N. Estimating the potential total number of novel Coronavirus cases in Wuhan City, China. Imperial College London; 2020.

143. Zhao S, Musa SS, Lin Q, Ran J, Yang G, Wang W, et al. Estimating the unreported number of novel coronavirus (2019-nCoV) cases in China in the first half of January 2020: a data-driven modelling analysis of the early outbreak. J Clin Med Res. 2020;9(2):388.

144. Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. Lancet. 2020 Feb 29;395(10225):689–97.

145. Riou J, Althaus CL. Pattern of early human-to-human transmission of Wuhan 2019 novel coronavirus (2019-nCoV), December 2019 to January 2020. Euro Surveill. 2020 Jan;25(4).

146. Oberholtzer K, Sivitz L, Mack A, Lemon S, Mahmoud A, Knobler S, editors. Learning from SARS: Preparing for the Next Disease Outbreak: Workshop Summary. National

Academies Press; 2004 Apr 26.

147. Fraser C, Riley S, Anderson RM, Ferguson NM. Factors that make an infectious disease outbreak controllable. Proc Natl Acad Sci U S A. 2004 Apr 20;101(16):6146–51.

148. Nsubuga P, White ME, Thacker SB, Anderson MA, Blount SB, Broome CV, Chiller TM, Espitia V, Imtiaz R, Sosin D, Stroup DF. Public health surveillance: a tool for targeting and monitoring interventions. Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; New York: Oxford University Press; 2006.

149. Republic of Argentina. Ficha de investigación caso de etiología desconocida [Case of unknown aetiology investigation form]. Argentina: Ministerio de Salud; 2021 Nov.

150. Russian Federation. ПРОФИЛАКТИКА ИНФЕКЦИОННЫХ БОЛЕЗНЕЙ [Prevention Of Infectious Diseases]. MU No.3.1.3114 /1-13. Russia: Ministry of Justice; 2013 Oct.

151. Republic of Liberia. Public Health Law Of Liberia; Revised (2019). Liberia; 2019.

152. Republic of Senegal. National Technical Guide For Integrated Disease Surveillance And Response. Senegal; 2019.

153. Sultanate of Oman Ministry of Health. Manual on Communicable Diseases. 3rd Edition. Oman; 2017.

154. Center for Disease Prevention and Control Latvia. Ziņošana par grupveida saslimšanas gadījumiem [Reporting of cluster outbreaks]. Latvia; 2020.

155. Ministry of Health Chile. Vigilancia de Enfermedades Transmisibles de Notificación Obligatoria [Surveillance of Communicable Diseases of Compulsory Notification]. Chile; 2022 Aug.

156. Integrated Disease Surveillance Project India. Format for instantaneous reporting of Early Warning Signal / outbreaks as soon as it is detected. India; 2022.

157. Royal Center for Disease Control. National Early Warning, Alert & Response Surveillance. Dorji T, editor. Royal Center for Disease Control, Ministry of Health Bhutan; 2015.

158. People's Republic of China. 突发公共卫生事件应急条例 [Emergency Regulations for Public Health Emergencies]. China: State Council; 2003 May.

159. Ministry of Health China. National Surveillance, Screening and Management Plan for Unknown Pneumonia Cases. Health Emergency No 158. China;2007.

160. Santos PD, Ziegler U, Szillat KP, Szentiks CA, Strobel B, Skuballa J, et al. In action—an early warning system for the detection of unexpected or novel pathogens. Virus Evolution. 2021 Oct 25;7(2):veab085.

161. Govender KN. Precision Pandemic Preparedness: Improving Diagnostics with Metagenomics. J Clin Microbiol. 2021 May 19;59(6).

162. Daughton CG. Wastewater surveillance for population-wide Covid-19: The present and future. Sci Total Environ. 2020 Sep 20;736:139631.

163. Arruda AG, Poljak Z, Knowles D, McLean A. Development of a stochastic agent-based model to evaluate surveillance strategies for detection of emergent porcine reproductive and respiratory syndrome strains. BMC Vet Res. 2017 Jun 12;13(1):171.

164. Hadorn DC, Stärk KDC. Evaluation and optimization of surveillance systems for rare and emerging infectious diseases. Vet Res. 2008 Jul 25;39(6):57.

165. Cameron AR, Meyer A, Faverjon C, Mackenzie C. Quantification of the sensitivity of early detection surveillance. Transbound Emerg Dis. 2020 Nov;67(6):2532–43.

166. Sah P, Fitzpatrick MC, Zimmer CF, Abdollahi E, Juden-Kelly L, Moghadas SM, et al. Asymptomatic SARS-CoV-2 infection: A systematic review and meta-analysis. Proc Natl Acad Sci U S A. 2021 Aug 24;118(34).

167. Goërtz YMJ, Van Herck M, Delbressine JM, Vaes AW, Meys R, Machado FVC, et al. Persistent symptoms 3 months after a SARS-CoV-2 infection: the post-COVID-19 syndrome? ERJ Open Res. 2020 Oct;6(4).

168. Gillespie DT. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. J Comput Phys. 1976 Dec 1;22(4):403–34.

169. Widgren S, Bauer P, Eriksson R, Engblom S. SimInf: An R package for data-driven stochastic disease spread simulations. J Stat Softw. 2019;91(12).

170. United Nations. Wuhan, China Metro Area Population 1950-2023 [Internet]. Macrotrends. 2023 [cited 2023 Mar 31].

171. Bjørnstad ON. Epidemics: Models and Data Using R. Springer Nature; 2022.

172. Byrne AW, McEvoy D, Collins AB, Hunt K, Casey M, Barber A, et al. Inferred duration of infectious period of SARS-CoV-2: rapid scoping review and analysis of available evidence for asymptomatic and symptomatic COVID-19 cases. BMJ Open. 2020 Aug 5;10(8):e039856.

173. Faes C, Abrams S, Van Beckhoven D, Meyfroidt G, Vlieghe E, Hens N, et al. Time between Symptom Onset, Hospitalisation and Recovery or Death: Statistical Analysis of Belgian COVID-19 Patients. Int J Environ Res Public Health. 2020 Oct 17;17(20).

174. Ayoub HH, Mumtaz GR, Seedat S, Makhoul M, Chemaitelly H, Abu-Raddad LJ. Estimates of global SARS-CoV-2 infection exposure, infection morbidity, and infection mortality rates in 2020. Glob Epidemiol. 2021 Nov;3:100068.

175. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 28;395(10229):1054–62.

176. Linton NM, Kobayashi T, Yang Y, Hayashi K, Akhmetzhanov AR, Jung SM, et al. Incubation period and other epidemiological characteristics of 2019 novel coronavirus infections with right truncation: a statistical analysis of publicly available case data. J

Clin Med Res. 2020;9(2):538.

177. Macedo A, Gonçalves N, Febra C. COVID-19 fatality rates in hospitalized patients: systematic review and meta-analysis. Ann Epidemiol. 2021 May;57:14–21.

178. Zhao S, Department of Applied Mathematics, Hong Kong Polytechnic University, Kong H, China, School of Nursing, et al. Estimating the time interval between transmission generations when negative values occur in the serial interval data: using COVID-19 as an example. Vol. 17, Mathematical Biosciences and Engineering. 2020. p. 3512–9.

179. Casey-Bryars M, Griffin J, McAloon C, Byrne A, Madden J, Mc Evoy D, et al. Presymptomatic transmission of SARS-CoV-2 infection: a secondary analysis using published data. BMJ Open. 2021 Jun 28;11(6):e041240.

180. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci. 2020 May;63(5):706–11.

181. Delamater PL, Street EJ, Leslie TF, Yang YT, Jacobsen KH. Complexity of the Basic Reproduction Number (R0). Emerg Infect Dis. 2019 Jan;25(1):1–4.

182. Mercer GN, Glass K, Becker NG. Effective reproduction numbers are commonly overestimated early in a disease outbreak. Stat Med. 2011 Apr 30;30(9):984–94.

183. Nicholson WL. On the Normal Approximation to the Hypergeometric Distribution. Ann Math Stat. 1956;27(2):471–83.

184. Bentley JL. Multidimensional divide-and-conquer. Commun ACM. 1980 Apr 1;23(4):214–29.

185. Huisman JS, Scire J, Angst DC, Li J, Neher RA, Maathuis MH, et al. Estimation and worldwide monitoring of the effective reproductive number of SARS-CoV-2. Elife. 2022 Aug 8;11.

186. Koo JR, Cook AR, Park M, Sun Y, Sun H, Lim JT, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. Lancet Infect Dis. 2020 Jun;20(6):678–88.

187. Wu WK, Liou JM, Hsu CC, Lin YH, Wu MS. Pandemic preparedness in Taiwan. Nat Biotechnol. 2020 Aug;38(8):932–3.

188. Ahmad J, Ahmad M, Usman ARA, Al-Wabel MI. Prevalence of human pathogenic viruses in wastewater: A potential transmission risk as well as an effective tool for early outbreak detection for COVID-19. J Environ Manage. 2021 Nov 15;298:113486.

189. Abdeldayem OM, Dabbish AM, Habashy MM, Mostafa MK, Elhefnawy M, Amin L, et al. Viral outbreaks detection and surveillance using wastewater-based epidemiology, viral air sampling, and machine learning techniques: A comprehensive review and outlook. Sci Total Environ. 2022 Jan 10;803:149834.

190. Corpuz MVA, Buonerba A, Vigliotta G, Zarra T, Ballesteros F Jr, Campiglia P, et al. Viruses in wastewater: occurrence, abundance and detection methods. Sci Total Environ.

2020 Nov 25;745:140910.

191. Böttcher L, D'Orsogna MR, Chou T. A statistical model of COVID-19 testing in populations: effects of sampling bias and testing errors. Philosophical Transactions of the Royal Society A. 2022 Jan 10;380(2214):20210121.

192. Flaxman S, Mishra S, Gandy A, Unwin HJT, Mellan TA, Coupland H, et al. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. Nature. 2020 Aug;584(7820):257–61.

193. Dhillon RS, Srikrishna D. When is contact tracing not enough to stop an outbreak? Lancet Infect Dis. 2018 Dec 1;18(12):1302–4.

194. Tupper P, Otto SP, Colijn C. Fundamental limitations of contact tracing for COVID-19. Facets. 2021 Jan 1;6:1993–2001.

195. Pouwels KB, House T, Pritchard E, Robotham JV, Birrell PJ, Gelman A, et al. Community prevalence of SARS-CoV-2 in England from April to November, 2020: results from the ONS Coronavirus Infection Survey. The Lancet Public Health. 2021 Jan 1;6(1):e30–8.

196. Lillie PJ, Samson A, Li A, Adams K, Capstick R, Barlow GD, et al. Novel coronavirus disease (Covid-19): The first two patients in the UK with person to person transmission. J Infect. 2020 May;80(5):578–606.

197. Iacobucci G. Covid-19: What is the UK's testing strategy? BMJ. 2020 Mar 26;368.

198. Flaxman S, Mishra S, Gandy A, Unwin H, Coupland H, Mellan T, et al. Estimating the number of infections and the impact of non-pharmaceutical interventions on COVID-19 in 11 European countries. 2020 Mar 30;13.

199. Jit M, Jombart T, Nightingale ES, Endo A, Abbott S, LSHTM Centre for Mathematical Modelling of Infectious Diseases COVID-19 Working Group, et al. Estimating number of cases and spread of coronavirus disease (COVID-19) using critical care admissions, United Kingdom, February to March 2020. Euro Surveill. 2020 May;25(18).

200. Brown J, Kirk-Wade E. Coronavirus: A History of "Lockdown Laws" in England. House of Commons Library; 2021 Dec 22. Report No.9068.

201. World Health Organization. Surveillance case definitions for human infection with novel coronavirus (nCoV), interim guidance. World Health Organization; 2020 Jan 15. License: CC BY-NC-SA 3.0 IGO.

202. Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, et al. Assay Techniques and Test Development for COVID-19 Diagnosis. ACS Cent Sci. 2020 May 27;6(5):591–605.

203. Surkova E, Nikolayevskyy V, Drobniewski F. False-positive COVID-19 results: hidden problems and costs. Lancet Respir Med. 2020 Dec;8(12):1167–8.

204. UK Health Security Agency. COVID-19: investigation and initial clinical management of possible cases [Internet]. 2020 [cited 2020 Apr 15].

205. Ma H, Zeng W, He H, Zhao D, Jiang D, Zhou P, et al. Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol. 2020 Jul;17(7):773–5.

206. Ernst E, Wolfe P, Stahura C, Edwards KA. Technical considerations to development of serological tests for SARS-CoV-2. Talanta. 2021 Mar 1;224:121883.

207. Lewandowski K, Xu Y, Pullan ST, Lumley SF, Foster D, Sanderson N, et al. Metagenomic Nanopore Sequencing of Influenza Virus Direct from Clinical Respiratory Samples. J Clin Microbiol. 2019 Dec 23;58(1).

208. Peeling RW, Wedderburn CJ, Garcia PJ, Boeras D, Fongwen N, Nkengasong J, Sall A, Tanuri A, Heymann DL. Serology testing in the COVID-19 pandemic response. The Lancet Infectious Diseases. 2020 Sep 1;20(9):e245-9.

209. Hart WS, Maini PK, Thompson RN. High infectiousness immediately before COVID-19 symptom onset highlights the importance of continued contact tracing. Elife. 2021 Apr 26;10.

210. Mahase E. Covid-19: UK regulator approves lateral flow test for home use despite accuracy concerns. BMJ. 2020 Dec 23;371:m4950.

211. Alene M, Yismaw L, Assemie MA, Ketema DB, Mengist B, Kassie B, et al. Magnitude of asymptomatic COVID-19 cases throughout the course of infection: A systematic review and meta-analysis. PLoS One. 2021 Mar 23;16(3):e0249090.

212. Lovell-Read FA, Funk S, Obolski U, Donnelly CA, Thompson RN. Interventions targeting non-symptomatic cases can be important to prevent local outbreaks: SARS-CoV-2 as a case study. Journal of the Royal Society Interface. 2021 May 19;18(178):20201014.

213. Byambasuren O, Cardona M, Bell K, Clark J, McLaws ML, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. J Assoc Med Microbiol Infect Dis Can. 2020 Dec;5(4):223–34.

214. Yanes-Lane M, Winters N, Fregonese F, Bastos M, Perlman-Arrow S, Campbell JR, et al. Proportion of asymptomatic infection among COVID-19 positive persons and their transmission potential: A systematic review and meta-analysis. PLoS One. 2020 Nov 3;15(11):e0241536.

215. Meyerowitz EA, Richterman A, Bogoch II, Low N, Cevik M. Towards an accurate and systematic characterisation of persistently asymptomatic infection with SARS-CoV-2. Lancet Infect Dis. 2021 Jun;21(6):e163–9.

216. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus symptomatic COVID-19. Lancet. 2021 Jan 9;397(10269):93–4.

217. Whittaker RH. Evolution and measurement of species diversity. Taxon. 1972 May;21(2-3):213–51.

218. Wu Y, Cheng X, Jiang G, Tang H, Ming S, Tang L, et al. Altered oral and gut microbiota and its association with SARS-CoV-2 viral load in COVID-19 patients during

hospitalization. NPJ Biofilms Microbiomes. 2021 Jul 22;7(1):61.

219. Gauthier NPG, Locher K, MacDonald C, Chorlton SD, Charles M, Manges AR. Alterations in the nasopharyngeal microbiome associated with SARS-CoV-2 infection status and disease severity. PLoS One. 2022 Oct 14;17(10):e0275815.

220. Gupta A, Bhanushali S, Sanap A, Shekatkar M, Kharat A, Raut C, et al. Oral dysbiosis and its linkage with SARS-CoV-2 infection. Microbiol Res. 2022 Aug;261:127055.

221. Rafiqul Islam SM, Foysal MJ, Hoque MN, Mehedi HMH, Rob MA, Salauddin A, et al. Dysbiosis of Oral and Gut Microbiomes in SARS-CoV-2 Infected Patients in Bangladesh: Elucidating the Role of Opportunistic Gut Microbes. Front Med. 2022 Feb 14;9:821777.

222. Feng S, Shen C, Xia N, Song W, Fan M, Cowling BJ. Rational use of face masks in the COVID-19 pandemic. Vol. 8, The Lancet Respiratory Medicine. 2020. p. 434–6.

223. Fancourt D, Bu F, Mak HW, Steptoe A. COVID-19 social study: Results release 2. Department of Behavioural Science & Health: University College London. 2020 Apr 2.

224. Wright L, Steptoe A, Fancourt D. Predictors of self-reported adherence to COVID-19 guidelines. A longitudinal observational study of 51,600 UK adults. Lancet Reg Health Eur. 2021 May;4:100061.

225. Burton A, McKinlay A, Dawes J, Roberts A, Fynn W, May T, Fancourt D. Understanding barriers and facilitators to compliance with UK social distancing guidelines during the COVID-19 pandemic: A qualitative interview study. Behaviour Change. 2023 Apr;40(1):30-50.

226. Naing L, Winn T, Rusli BN. Practical issues in calculating the sample size for prevalence studies. Archives of orofacial Sciences. 2006;1:9–14.

227. Kinross P, Suetens C, Gomes Dias J, Alexakis L, Wijermans A, Colzani E, et al. Rapidly increasing cumulative incidence of coronavirus disease (COVID-19) in the European Union/European Economic Area and the United Kingdom, 1 January to 15 March 2020. Euro Surveill. 2020 Mar;25(11).

228. Nunes-Vaz R. Visualising the doubling time of COVID-19 allows comparison of the success of containment measures. Global Biosecurity. 2020;2(1).

229. Office for National Statistics. Coronavirus (COVID-19) Infection Survey: England [Internet]. Office for National Statistics; 2022 [cited 2022 Dec 11].

230. Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Acad Emerg Med. 1996 Sep;3(9):895–900.

231. Office for National Statistics. Population estimates by single year of age and sex for local authorities in the United Kingdom: 2011 Census [Internet]. 2011 [cited 2022 Dec 13].

232. Uschner D, Schindler D, Hilgers RD, Heussen N. randomizeR: An R Package for the Assessment and Implementation of Randomization in Clinical Trials. J Stat Softw. 2018 Jun 19;85:1–22.

233. Mamouris P, Nassiri V, Molenberghs G, van den Akker M, van der Meer J, Vaes B. Fast and optimal algorithm for case-control matching using registry data: application on the antibiotics use of colorectal cancer patients. BMC Med Res Methodol. 2021 Apr 2;21(1):62.

234. Claro IM, Ramundo MS, Coletti TM, da Silva CAM, Valenca IN, Candido DS, et al. Rapid viral metagenomics using SMART-9N amplification and nanopore sequencing. Wellcome Open Res. 2021 Sep 20;6:241.

235. Fan J, Huang S, Chorlton SD. BugSeq: a highly accurate cloud platform for long-read metagenomic analyses. BMC Bioinformatics. 2021 Mar 25;22(1):160.

236. Gauthier NPG, Nelson C, Bonsall MB, Locher K, Charles M, MacDonald C, et al. Nanopore metagenomic sequencing for detection and characterization of SARS-CoV-2 in clinical samples. PLoS One. 2021 Nov 18;16(11):e0259712.

237. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018 Sep 15;34(18):3094–100.

238. McSweeney R. Coronavirus and key workers in the UK [Internet]. Office for National Statistics; 2020 [cited 2022 Dec 21].

239. National Health Service. Coronavirus (COVID-19): What is self-isolation and why is it important? [Internet]. 2020 [cited 2022 Dec 29].

240. Sherrill-Mix S. taxonomizr: Functions to Work with NCBI Accessions and Taxonomy. R package version 0.5. 3. 2019.

241. Sakamoto T, Ortega JM. Taxallnomy: an extension of NCBI Taxonomy that produces a hierarchically complete taxonomic tree. BMC Bioinformatics. 2021 Jul 29;22(1):388.

242. Aranha C, Patel V, Bhor V, Gogoi D. Cycle threshold values in RT-PCR to determine dynamics of SARS-CoV-2 viral load: An approach to reduce the isolation period for COVID-19 patients. J Med Virol. 2021 Dec;93(12):6794–7.

243. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PMM. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol. 2003 Nov;56(11):1129–35.

244. Halkin A, Reichman J, Schwaber M, Paltiel O, Brezis M. Likelihood ratios: getting diagnostic testing into perspective. QJM. 1998 Apr;91(4):247–58.

245. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc. 1995 Jan;57(1):289–300.

246. Benjamini Y. Discovering the false discovery rate. J R Stat Soc Series B Stat Methodol. 2010 Aug 5;72(4):405–16.

247. Clopper CJ, Pearson ES. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika. 1934;26(4):404–13.

248. Mercaldo ND, Lau KF, Zhou XH. Confidence intervals for predictive values with an emphasis to case-control studies. Stat Med. 2007 May 10;26(10):2170–83.

249. Fox J, Weisberg S. An R Companion to Applied Regression. SAGE Publications; 2018.

250. Emura T, Liao YT. Critical review and comparison of continuity correction methods: The normal approximation to the binomial distribution. Communications in Statistics - Simulation and Computation. 2018 Sep 14;47(8):2266–85.

251. Camilli G, Hopkins KD. Applicability of chi-square to 2 × 2 contingency tables with small expected cell frequencies. Psychol Bull. 1978 Jan 19;85(1):163–7.

252. Ruxton GD, Neuhäuser M. Good practice in testing for an association in contingency tables. Behav Ecol Sociobiol. 2010 Sep;64(9):1505–13.

253. Shannon CE. A mathematical theory of communication. The Bell System Technical Journal. 1948 Jul;27(3):379–423.

254. Oksanen J. Vegan: Community Ecology Package [Internet]. R package version 1.8-5. 2007.

255. Gloor GB, Macklaim JM, Fernandes AD. Displaying Variation in Large Datasets: Plotting a Visual Summary of Effect Sizes. J Comput Graph Stat. 2016 Jul 2;25(3):971–9.

256. Office for National Statistics. Oxfordshire County: Local Area Report [Internet]. Nomis: Official Census and Labour Market Statistics. 2011 [cited 2022 Dec 19].

257. Office for National Statistics. Oxford Local Authority: Local Area Report [Internet]. Nomis: Official Census and Labour Market Statistics. 2011 [cited 2022 Dec 19].

258. Office for National Statistics. Coronavirus (COVID-19) infection survey: England [Internet]. Office for National Statistics. 2020 [cited 2022 Dec 7].

259. Van Elslande J, Houben E, Depypere M, Brackenier A, Desmet S, André E, et al. Diagnostic performance of seven rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. Clin Microbiol Infect. 2020 Aug;26(8):1082–7.

260. Lutalo T, Nalumansi A, Olara D, Kayiwa J, Ogwang B, Odwilo E, et al. Evaluation of the performance of 25 SARS-CoV-2 serological rapid diagnostic tests using a reference panel of plasma specimens at the Uganda Virus Research Institute. Int J Infect Dis. 2021 Nov 1;112:281–7.

261. Owen SI, Williams CT, Garrod G, Fraser AJ, Menzies S, Baldwin L, et al. Twelve lateral flow immunoassays (LFAs) to detect SARS-CoV-2 antibodies. J Infect. 2022 Mar;84(3):355–60.

262. Ondov BD, Bergman NH, Phillippy AM. Interactive metagenomic visualization in a Web browser. BMC Bioinformatics. 2011 Sep 30;12(1):385.

263. Office for National Statistics. Coronavirus (COVID-19) Infection Survey: England

[Internet]. Office for National Statistics. 2022 [cited 2022 Oct 19].

264. Mégarbane B, Bourasset F, Scherrmann JM. Is Lockdown Effective in Limiting SARS-CoV-2 Epidemic Progression?-a Cross-Country Comparative Evaluation Using Epidemiokinetic Tools. J Gen Intern Med. 2021 Mar;36(3):746–52.

265. Nuertey BD, Ekremet K, Haidallah AR, Mumuni K, Addai J, Attibu RIE, et al. Performance of COVID-19 associated symptoms and temperature checking as a screening tool for SARS-CoV-2 infection. PLoS One. 2021 Sep 17;16(9):e0257450.

266. Paradiso AV, De Summa S, Loconsole D, Procacci V, Sallustio A, Centrone F, et al. Rapid Serological Assays and SARS-CoV-2 Real-Time Polymerase Chain Reaction Assays for the Detection of SARS-CoV-2: Comparative Study. J Med Internet Res. 2020 Oct 30;22(10):e19152.

267. Seaman CP, Tran LTT, Cowling BJ, Sullivan SG. Self-collected compared with professional-collected swabbing in the diagnosis of influenza in symptomatic individuals: A meta-analysis and assessment of validity. J Clin Virol. 2019 Sep;118:28–35.

268. Wang H, Liu Q, Hu J, Zhou M, Yu MQ, Li KY, et al. Nasopharyngeal Swabs Are More Sensitive Than Oropharyngeal Swabs for COVID-19 Diagnosis and Monitoring the SARS-CoV-2 Load. Front Med. 2020 Jun 18;7:334.

269. Gertler M, Krause E, van Loon W, Krug N, Kausch F, Rohardt C, et al. Self-collected oral, nasal and saliva samples yield sensitivity comparable to professionally collected oro-nasopharyngeal swabs in SARS-CoV-2 diagnosis among symptomatic outpatients. Int J Infect Dis. 2021 Sep 1;110:261–6.

270. D'Andrea EL, Cossu AM, Scrima M, Messina V, Iuliano P, Di Perna F, et al. Efficacy of Unsupervised Self-Collected Mid-Turbinate FLOQSwabs for the Diagnosis of Coronavirus Disease 2019 (COVID-19). Viruses. 2021 Aug 22;13(8).

271. Jordan RE, Adab P. Who is most likely to be infected with SARS-CoV-2? Lancet Infect Dis. 2020 Sep;20(9):995–6.

272. Borkakoty B, Bali NK. Haemophilus influenzae and SARS-CoV-2: Is there a role for investigation? Indian J Med Microbiol. 2021 Apr;39(2):240–4.

273. Scharff RL, Viscusi WK. Heterogeneous rates of time preference and the decision to smoke. Econ Inq. 2011;49(4):959–72.

274. Lloyd A, McKay R, Hartman TK, Vincent BT, Murphy J, Gibson-Miller J, et al. Delay discounting and under-valuing of recent information predict poorer adherence to social distancing measures during the COVID-19 pandemic. Sci Rep. 2021 Sep 28;11(1):19237.

275. Ding X, Brazel DM, Mills MC. Factors affecting adherence to non-pharmaceutical interventions for COVID-19 infections in the first year of the pandemic in the UK. BMJ Open. 2021 Oct 25;11(10):e054200.

276. Smith LE, Amlôt R, Lambert H, Oliver I, Robin C, Yardley L, et al. Factors associated

with adherence to self-isolation and lockdown measures in the UK: a cross-sectional survey. Public Health. 2020 Oct;187:41–52.

277. Timmons S, McGinnity F, Belton C, Barjaková M, Lunn P. It depends on how you ask: measuring bias in population surveys of compliance with COVID-19 public health guidance. J Epidemiol Community Health. 2021 Apr 1;75(4):387-9.

278. Wu JS, Font X, McCamley C. COVID-19 social distancing compliance mechanisms: UK evidence. Environ Res. 2022 Apr 1;205:112528.

279. Masters R, Anwar E, Collins B, Cookson R, Capewell S. Return on investment of public health interventions: a systematic review. J Epidemiol Community Health. 2017 Aug;71(8):827–34.

280. Forman R, Azzopardi-Muscat N, Kirkby V, Lessof S, Nathan NL, Pastorino G, et al. Drawing light from the pandemic: Rethinking strategies for health policy and beyond. Health Policy. 2022 Jan;126(1):1–6.

281. Norzin T, Ghiasbeglou H, Patricio M, Romanova S, Zaghlool A, Tanguay F, et al. Event-based surveillance: Providing early warning for communicable disease threats. Can Commun Dis Rep. 2023;49.

282. Dulanto Chiang A, Dekker JP. From the Pipeline to the Bedside: Advances and Challenges in Clinical Metagenomics. J Infect Dis. 2020 Mar 28;221(S3):S331–40.

283. Chen X, Zhou T, Feng L, Liang J, Liljeros F, Havlin S, et al. Nontrivial resource requirement in the early stage for containment of epidemics. Phys Rev E. 2019 Sep 23;100(3):032310.

284. Kaul KL. Laboratories and Pandemic Preparedness: A Framework for Collaboration and Oversight. J Mol Diagn. 2020 Jul;22(7):841–3.

285. Donia A, Hassan SU, Zhang X, Al-Madboly L, Bokhari H. COVID-19 Crisis Creates Opportunity towards Global Monitoring & Surveillance. Pathogens. 2021 Feb 24;10(3).

286. Pasteur L. The germ theory and its application to medicine and surgery [translated by Harold C. Ernst]. Scientific Papers (Harvard Classics Vol 38) New York. 1910;38:382–9.

287. Economist Impact. GHS Index Methodology [Internet]. Nuclear Threat Initiative; 2021 Dec.

288. World Health Organization. Joint external evaluation tool: International Health Regulations (2005), third edition. Geneva: World Health Organization; 2022.

289. World Legal Information Institute. World Legal Information Institute [Internet]. 2023 [cited 2023 Jan 3].

290. VERTIC - The Verification Research, Training and Information Centre [Internet]. Vertic. 2016 [cited 2023 Jan 5].

291. International Labour Organization. NATLEX Database of national labour, social security

and related human rights legislation [Internet]. 2023 [cited 2023 Jan 23].

292. Pacific public health surveillance network. Pacific Public Health Surveillance Network [Internet]. 2022 [cited 2023 Jan 5].

293. Pan American Health Organization. Pan American Health Organization [Internet]. 2022 [cited 2023 Jan 5].

294. The Caribbean Public Health Agency (CARPHA). The Caribbean Public Health Agency [Internet]. 2022 [cited 2023 Jan 5].

295. European Centre for Disease Prevention and Control (ECDC). European Centre for Disease Prevention and Control [Internet]. 2022 [cited 2023 Jan 5].

296. World Health Organization. Yearbook of the United Nations 1984. United Nations; 1984. p. 1220–8.

297. Google. Google Translate [Internet]. 2023 [cited 2023 Jan 5].

298. Balk EM, Chung M, Hadar N, Patel K, Yu WW, Trikalinos TA, et al. Accuracy of Data Extraction of Non-English Language Trials with Google Translate. Rockville (MD): Agency for Healthcare Research and Quality (US);

299. Jackson JL, Kuriyama A, Anton A, Choi A, Fournier JP, Geier AK, et al. The Accuracy of Google Translate for Abstracting Data From Non–English-Language Trials for Systematic Reviews. Ann Intern Med. 2019 Nov 5;171(9):677–9.

300. World Health Organization. Joint External Evaluation (JEE) [Internet]. World Health Organization; 2023 [cited 2023 Jan 16].

301. Skowron K, Bauza-Kaszewska J, Grudlewska-Buda K, Wiktorczyk-Kapischke N, Zacharski M, Bernaciak Z, et al. Nipah Virus–Another Threat From the World of Zoonotic Viruses. Front Microbiol. 2022;12.

302. WHO Team Emergency Response. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003 [Internet]. World Health Organisation. 2015 [cited 2023 Jan 29].

303. World Bank. Current health expenditure (% of GDP) [Internet]. The World Bank Group. 2022 [cited 2023 Feb 15].

304. World Bank. Domestic general government health expenditure (% of GDP) [Internet]. The World Bank Group. 2022 [cited 2023 Feb 15].

305. World Bank. Population, total [Internet]. The World Bank Group. 2022 [cited 2023 Feb 15].

306. World Bank. GDP per capita (current US$) [Internet]. The World Bank Group. 2022 [cited 2023 Feb 15].

307. World Bank. GDP (current US$) [Internet]. The World Bank Group. 2022 [cited 2023 Feb 15].

308. Fall IS, Rajatonirina S, Yahaya AA, Zabulon Y, Nsubuga P, Nanyunja M, et al. Integrated Disease Surveillance and Response (IDSR) strategy: current status, challenges and perspectives for the future in Africa. BMJ Glob Health. 2019 Jul 3;4(4):e001427.

309. United Nations. Human Development Index [Internet]. United Nations Development Programme; 2022 [cited 2023 Feb 15].

310. World Health Organization. International Health Regulations (2005): State Party Self-assessment annual reporting tool, second edition. Geneva: World Health Organization; 2021.

311. Islamic Republic of Afghanistan. Ministry of Public Health [Internet]. Afghanistan; 2022 [cited 2022 Jul 1].

312. World Health Organization. Joint external evaluation of IHR Core Capacities of the Islamic Republic of Afghanistan, Mission report: 4- 7 December 2016. No. WHO/WHE/CPI/REP/2017.43. Geneva: World Health Organization; 2017

313. Islamic Republic of Afghanistan. Afghanistan National Health Policy 2015-2020. Afghanistan; 2015 Nov.

314. Islamic Republic of Afghanistan. Public Health Law No 97. Afghanistan; 2007 Jan.

315. Ministry of Public Health. NDSR Weekly Epidemiological Report 47 [Internet]. Afghanistan; 2022 Nov [cited 2023 Jan 5].

316. Ministry of Health. Ministria e Shëndetësisë dhe Mbrojtjes Sociale [Ministry of Health and Social Protection] [Internet]. Republic of Albania. 2022 [cited 2022 Jul 17].

317. World Health Organization. Joint external evaluation of IHR Core Capacities of the Republic of Albania, Mission report: September 2016. No. WHO/WHE/CPI/2017.18. Geneva: World Health Organization; 2017.

318. Republic of Albania. Për Shëndetin Publik [Public Health Law]. Albania; 2009 May.

319. Republic of Albania. Për Parandalimin Dhe Luftimin E Infeksioneve Dhe Sëmundjeve Infektive [For the prevention and fight against infections and infectious diseases]. Vol. 46. Albania; 2016 Mar.

320. Republic of Albania. COVID-19 Plan Veprimi Për Shqipërine [COVID-19 Action Plan For Albania]. Instituti i Shëndetit Publik: Ministria e Shëdetësisë dhe Mbrojtjes Sociale; 2020 Mar.

321. Republic of Albania. Incidenca e sëmundjeve infektive [Incidence of infectious diseases] [Internet]. Ministria e Shëndetësisë; 2018 Jun [cited 2022 Jul 20].

322. People's Democratic Republic of Algeria. Ministère de la Santé [Ministry of Health] [Internet]. République Algérienne Démocratique et Populaire; 2022 [cited 2022 Aug 9].

323. Ait-Mohand A. La déclaration obligatoire de certaines maladies infectieuses en Algérie [Mandatory notification of certain infectious diseases in Algeria]. J alger med. 2020 Jun 1;XXVIII(2):1–4.

324. People's Democratic Republic of Algeria. Loi à la protection et à la promotion de la santé [Law relating to the protection and promotion of health] 85-05. République Algérienne Démocratique Populaire; 1985 Feb.

325. Principality of Andorra. Salut [Health] [Internet]. Govern d'Andorra. 2022 [cited 2022 Aug 8].

326. Principality of Andorra. Pla Nacional Salut 2020 [National Health Plan 2020]. Andorra: Govern d'Andorra; 2020.

327. Principality of Andorra. General Health Law 63. Andorra: Govern d'Andorra; 2009 Sep.

328. Principality of Andorra. Vigilància de les malalties de declaració obligatòria 2016-2020 [Surveillance of diseases of mandatory declaration 2016-2020]. Govern d'Andorra; 2021 Jun.

329. Republic of Angola. Ministério da Saúde [Ministry of Health] [Internet]. 2022 [cited 2022 Aug 12].

330. World Health Organization. Joint external evaluation of IHR Core Capacities of the Republic of Angola, Mission report 18-22 November 2019. Geneva: World Health Organization; 2020.

331. Republic of Angola. Plano Nacional de Desenvolvimento Sanitário 2012-2025 [National Health Development Plan 2012-2025]. Vol 2. Ministério da Saúde; 2015 Jan.

332. Republic of Angola. Plano Nacional de Contingência Para o Controlo da Epidemia de Doença por Coronavírus 2019 (COVID-19) [National Contingency Plan for the Control of the Coronavirus Disease Epidemic 2019 (COVID-19)]. República de Angola: Ministério da Saúde; 2020 Feb.

333. Republic of Angola. Estatuto Orgânico Do Ministério Da Saude [Organic Statute Of The Ministry Of Health] Decreto Lei 2/00. Angola; 2000 Jan.

334. Antigua and Barbuda. Ministry of Health [Internet]. 2023 [cited 2023 Jan 5].

335. Antigua and Barbuda. Public Health Act. No 353. 2020 Jul.

336. Ministry of Health. COVID-19 Guidelines [Internet]. Government of Antigua and Barbuda. 2020 [cited 2020 Jul 18].

337. Republic of Argentina. Epidemiología [Epidemiology]. Argentina: Ministerio de Salud; 2022 Jul.

338. Republic of Argentina. Administración Nacional de Laboratorios e Institutos de Salud [National Administration of Laboratories and Health Institutes] [Internet]. Ministerio de Salud. 2022 [cited 2022 Aug 8].

339. Republic of Argentina. Public Health Law. No.15-465.Argentina; 1960 Oct.

340. Republic of Argentina. Ministerio De Salud Resolución [Ministry of Health Resolution]. No.2827/2022. Ministerio De Salud; 2022 Nov.

341. Ministry of Health. Manual De Normas Y Procedimientos De Vigilancia Y Control De Eventos De Notificación Obligatoria Actualización [Manual Of Standards And Procedures For Surveillance And Control Of Compulsory Notification Events]. Republic of Argentina; 2022 Nov.

342. Republic of Argentina. Sistema Nacional de Vigilancia de la Salud (SNVS) [National Health Surveillance System]. Argentina: Ministerio de Salud; 2018 Oct.

343. Republic of Argentina. Estrategia integrada de vigilancia de las infecciones respiratorias agudas de posible origen viral [Integrated surveillance strategy for acute respiratory infections of possible viral origin]. Argentina: Ministerio de Salud; 2022 May.

344. Republic of Argentina. Boletín Epidemiológico Nacional 51 [National Epidemiological Bulletin 51]. Ministerio de Salud; 2022 Dec.

345. Republic of Armenia. ՀԱՅԱՍՏԱՆԻ ՀԱՆՐԱՊԵՏՈՒԹՅԱՆ [Ministry of Health] [Internet]. Armenia; 2022 [cited 2022 Aug 9].

346. Republic of Armenia. Ministry of Health: National Center for Disease Control and Prevention [Internet]. Armenia; 2022 [cited 2022 Aug 9].

347. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Republic of Armenia, Mission report: 15-19 August 2016. No. WHO/WHE/CPI/2017.14. Geneva: World Health Organization; 2017.

348. Republic of Armenia. ՎԱՐԱԿԻԶ ՀԻՎԱՆԴՈՒԹՅՈՒՆՆԵՐԻ «ԻՐԱԿԱՆ ԺԱՄԱՆԱԿՈՒՄ» ԷԼԵԿՏՐՈՆԱՅԻՆ ՀԱՄԱՃԱՐԱԿԱԲԱՆԱԿԱՆ ՀՍԿՈՂՈՒԹՅՈՒՆ ՍԱՆԻՏԱՐԱՀԱՄԱՃԱՐԱԿԱՅԻՆ ՆՈՐՄԵՐԻ ԵՎ ԿԱՆՈՆՆԵՐԻ ՀԱՍՏԱՏՄԱՆ ՄԱՍԻՆ [Mechanisms of cooperation and coordination procedures of the national coordinating body and interested bodies in questions of international health (sanitary) rules]. Republic of Armenia: Ministry of Health; 2010 Dec.

349. Republic of Armenia. ՎԱՐԱԿԻԶ ՀԻՎԱՆԴՈՒԹՅՈՒՆՆԵՐԻ «ԻՐԱԿԱՆ ԺԱՄԱՆԱԿՈՒՄ» ԷԼԵԿՏՐՈՆԱՅԻՆ ՀԱՄԱՃԱՐԱԿԱԲԱՆԱԿԱՆ ՀՍԿՈՂՈՒԹՅՈՒՆ ՍԱՆԻՏԱՐԱՀԱՄԱՃԱՐԱԿԱՅԻՆ ՆՈՐՄԵՐԻ ԵՎ ԿԱՆՈՆՆԵՐԻ ՀԱՍՏԱՏՄԱՆ ՄԱՍԻՆ [Real time electronic epidemiology surveillance of infectious diseases sanitary and epidemiological norms and rules]. Republic of Armenia: Ministry of Health; 2010 Dec.

350. Republic of Armenia. ՄԱՐԴՈՒ, ՄԱՐԴՈՒ ԵՎ ԿԵՆԴԱՆԻՆԵՐԻ ՀԱՄԱՐ ԸՆԴՀԱՆՈՒՐ ՎԱՐԱԿԻԶ ՀԻՎԱՆԴՈՒԹՅԱՆ ԿԱՍԿԱԾ ՆԵՐԿԱՅԱՑՆՈՂ ԱՆՍՈՎՈՐ ԵՎ ԱՆՍՊԱՍԵԼԻ ԴԵՊՔԵՐԻ ՀԱՄԱՃԱՐԱԿԱԲԱՆԱԿԱՆ ՀՍԿՈՂՈՒԹՅԱՆ ԿԱԶՄԱԿԵՐՊՈՒՄ [Organization Of Epidemiological Surveillance Of Unusual And Unexpected Cases Of Suspected Common Infectious Disease For Humans, Humans And Animals]. Republic of Armenia: Ministry of Health; 2013 Dec.

351. Republic of Armenia. ՄԻՋԱԶԳԱՅԻՆ ԱՌՈՂՋԱՊԱՀԱԿԱՆ (ԲԺՇԿԱՍԱՆԻՏԱՐԱԿԱՆ) ԿԱՆՈՆՆԵՐԻ ՀԱՐՑԵՐՈՎ ԱԶԳԱՅԻՆ ՀԱՄԱԿԱՐԳՈՂ ՄԱՐՄՆԻ ԵՎ ՇԱՀԱԳՐԳԻՌ ՄԱՐՄԻՆՆԵՐԻ ՀԱՄԱԳՈՐԾԱԿՑՈՒԹՅԱՆ ՄԵԽԱՆԻԶՄՆԵՐԸ ԵՎ ՀԱՄԱԿԱՐԳՄԱՆ ԸՆԹԱՑԱԿԱՐԳԵՐԸ [Mechanisms of Cooperation And Coordination Procedures Of The National Coordinating Body And Interested Bodies In Questions Of International Health (Sanitary) Rules]. Republic of Armenia: Ministry of Health; 2010 Aug.

352. Commonwealth of Australia. Department of Health and Aged Care [Internet]. 2023 [cited 2023 Jan 6].

353. World Health Organization. Joint external evaluation of IHR Core Capacities of Australia, Mission report: 24 November-1 December 2017. No. WHO/WHE/CPI/REP/2018.8. Geneva: World Health Organization; 2018.

354. Government of Australia. National Health Security (National Notifiable Disease List) Instrument 2018. No.F2022C00605. Australia: Government of Australia; 2022.

355. Commonwealth of Australia. Biosecurity Act 2015. No.61-2015. Australia: Federal Register of Legislation; 2020 Apr.

356. Department of Health and Aged Care. National Notifiable Diseases Surveillance System (NNDSS) [Internet]. 2022 [cited 2023 Jan 6].

357. Australian Health Protection Principal Committee. Emergency Response Plan for Communicable Disease Incidents of National Significance. Australia: Australian Government Department of Health and Aged Care; 2016.

358. Commonwealth of Australia. National Notifiable Diseases Surveillance System (NNDSS) fortnightly reports – 28 November to 11 December 2022 [Internet]. Australian Government Department of Health and Aged Care. 2023 [cited 2023 Jan 6].

359. Republic of Austria. Epidemiologie übertragbarer Krankheiten in Österreich [Epidemiology of communicable diseases in Austria] [Internet]. Social Ministry. 2022 [cited 2022 Jul 22].

360. Republic of Austria. Bundesrecht konsolidiert: Gesamte Rechtsvorschrift für Epidemiegesetz 1950 [Consolidated Federal Law: Entire legislation for the Epidemic Act 1950]. Austria: Bundesministerium für Digitalisierung und Wirtschaftsstandort; 2022 Aug.

361. Federal Ministry of Social Affairs, Health, Care and Consumer Protection. Statistik meldepflichtiger Infektionskrankheiten 3. Quartal 2022 [Statistics of notifiable infectious diseases 3rd quarter 2022]. Austria; 2022 Oct.

362. El-Khatib Z, Taus K, Richter L, Allerberger F, Schmid D. A syndrome-based surveillance system for infectious diseases among asylum seekers in Austrian reception centers, 2015-2018: analysis of reported data. JMIR Public Health and Surveillance. 2019;5(1):e11465.

363. Ministry of Health. İCTİMAİ SƏHİYYƏ və İSLAHATLAR MƏRKƏZİ [Center For Public Health And Corrections] [Internet]. Republic of Azerbaijan. 2022 [cited 2022 Jul 17].

364. Rasulzade Z, Ismayilova R, Mursalova N, Veliyev T. Enhancement of disease reporting after implementation of the electronic integrated disease surveillance system in Azerbaijan. J Infec Dis Treat. 2019 Apr 22;5:1–2.

365. Republic of Azerbaijan. Əhalinin sağlamlığının qorunması haqqında [On Protection of the Health of the Population] [Internet]. 1997 Jun [cited 2022 Jul 29].

366. Azerbaijan RO. XÜSUSİ TƏHLÜKƏLİ İNFEKSİYALARA NƏZARƏT MƏRKƏZİ [Control Of Specially Dangerous Infections]. Azerbaijan: Ministry Of Health; 2020.

367. Commonwealth of the Bahamas. Ministry of Health [Internet]. Government of the Bahamas. 2023 [cited 2023 Jan 16].

368. Ministry of Health. Number Of New Cases Of Selected Reported Infectious Diseases Bahamas, 1994-2010 [Internet]. Bahamas; 2014 [cited 2022 Aug 1].

369. Commonwealth of the Bahamas. Health Services Health Rules [Internet]. Bahamas; 1935 Apr [cited 2023 Jan 5].

370. Kingdom of Bahrain. Ministry of Health [Internet]. 2023 [cited 2023 Jan 16].

371. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Bahrain, Mission report: 4-8 September 2016. No. WHO/WHE/CPI/2017.4. Geneva: World Health Organization; 2017.

372. Kingdom of Bahrain. Public Health: Incidences of Notifiable Communicable Diseases 2013 - 2020. Ministry of Health; 2022 May.

373. Kingdom of Bahrain. Public Health Law. No 34. Bahrain; 2018 Aug.

374. Government of the People's Republic of Bangladesh. Ministry of Health and Family Welfare [Internet]. 2023 [cited 2023 Jan 16].

375. World Health Organization. Joint external evaluation of IHR core capacities of the People's Republic of Bangladesh, Mission report: May 2016. No. WHO/HSE/GCR/2016.23. Geneva: World Health Organization; 2017.

376. Institute of Epidemiology, Disease Control and Research. National Public Health Laboratory Strategy for Infectious Diseases [Internet]. Bangladesh: People's Republic of Bangladesh; 2022 Apr [cited 2023 Jan 5].

377. Institute of Epidemiology, Disease Control & Research. List of Outbreak Investigations done by IEDCR in 2016 [Internet]. 2021 [cited 2023 Jan 16].

378. Ministry of Law, Justice and Parliamentary Affairs. সংক্রামক রোগ (প্রতিরোধ, নিয়ন্ত্রণ ও নির্মূল) আইন, ২০১৮ (২০১৮ সনের ৬১ নং আইন) [Communicable Diseases (Prevention, Control and Eradication) Act]. Bangladesh: People's Republic of Bangladesh; 2018 Nov.

379. Kalam MA. Priority Communicable Disease Surveillance (PCDS) in Bangladesh. Online J Public Health Inform. 2014;6(1).

380. Government of Barbados. Ministry Of Health And Wellness [Internet]. 2022 [cited 2022 Jul 15].

381. Government of Barbados. Health Services (Communicable And Notifiable Diseases) Regulations. No. 179. Barbados; 1969 Dec.

382. Barbados. Emergency Management (Covid 19) Order. Barbados; 2020.

383. Caribbean Public Health Agency. State of Public Health in the Caribbean Region 2014 - 2016. Port of Spain, Trinidad and Tobago: Caribbean Public Health Agency; 2017.

384. Republic of Belarus. Ministry of Health [Internet]. 2023 [cited 2023 Jan 16].

385. Republic of Belarus. Экстренное извещение об инфекционных заболеваниях [Emergency notification of infectious diseases]. Report No.976. Belarus: Ministry of Health; 2006 Dec.

386. Republic of Belarus. Republican Center for Hygiene, Epidemiology and Public Health [Internet]. 2022 [cited 2022 Jul 19].

387. Federal Public Service for Health. Health [Internet]. Belgium: 2023 [cited 2023 Jan 16].

388. Sciensano. Epistat Infectious Diseases Data Explorations & Visualizations [Internet]. Belgium: 2022 [cited 2022 Jul 19].

389. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Belgium, Mission report: 19-23 June 2017. No. WHO/WHE/CPI/REP/2017.37. Geneva: World Health Organization; 2017.

390. Sciensano. Déclaration des maladies transmissibles [Notification of Communicable Diseases] [Internet]. Belgium. 2021 [cited 2022 Jul 19].

391. Care and Health. Meldingsplichtige infectieziekten [Notifiable infectious diseases] [Internet]. Flanders Belgium. 2022 [cited 2022 Jul 19].

392. Common Community Commission Brussels. Notification of Communicable Diseases [Internet]. Belgium. 2023 [cited 2023 Jan 17].

393. Government of Belize. Ministry of Health and Wellness [Internet]. 2023 [cited 2023 Jan 16].

394. Government of Belize. Public Health Act. No. 40. Belize; 2000.

395. Republic of Benin. Ministry of Health [Internet]. 2022 [cited 2022 Aug 5].

396. World Health Organization. Joint External Evaluation Of IHR Core Capacities Of The Republic Of Benin, Mission report 21-26 May 2017. No. WHO/WHE/CPI/REP/2017.48. Geneva: World Health Organization; 2017.

397. Hounmanou YMG, Agonsanou MSS, Dougnon V, Vodougnon MHB, Achoh EM, Mohammed J, et al. The necessity of mobile phone technologies for public health surveillance in Benin. Advances in Public Health; 2016 Jan 1.

398. Kingdom of Bhutan. Ministry of Health [Internet]. 2023 [cited 2023 Jan 16].

399. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Bhutan, Mission report: 11-15 December 2017. No. WHO/WHE/CPI/REP/2018.7. Geneva: World Health Organization; 2017.

400. Ministry of Health. Bhutan Pandemic Preparedness and Response Plan (BPPRP) and

Standard Operating Procedures. Bhutan: Kingdom of Bhutan; 2020.

401. Kingdom of Bhutan. Monthly Disease Epidemiology Report, December 2022 [Internet]. Royal Centre for Disease Control. 2023 [cited 2023 Jan 16].

402. World Health Organization. The Kingdom of Bhutan Health System Review. Bhutan: Asia Pacific Observatory on Health Systems and Policies; 2017. Report No.7-2.

403. Kingdom of Bhutan. National Health Policy [Internet]. Ministry of Health; 2015 Nov [cited 2023 Jan 16].

404. Bolivia. Ministry of Health and Sports [Internet]. 2023 [cited 2023 Jan 16].

405. Ministry of Health. Epidemiological Surveillance Bulletin [Internet]. Bolivia; 2018 Apr [cited 2023 Jan 16]. Report No.14.

406. Ministry of Health and Sports Bolivia. Supreme Decrees [Internet]. 2023 [cited 2023 Jan 16].

407. Federation of Bosnia and Herzegovina. Federal Ministry of Health [Internet]. 2023 [cited 2023 Jan 16].

408. The Institute for Public Health of the Federation of Bosnia and Herzegovina. Epidemiology Service [Internet]. 2022 [cited 2022 Aug 11].

409. Institute for Public Health. Godišnji Izvještaj O Zaraznim Bolestima I Provedenim Imunizacijama U Federaciji Bosne I Hercegovine U 2020. Godini [Annual Report On Infectious Diseases I Immunizations Performed In The Federation Bosnia And Herzegovina In 2020] [Internet]. Federation Bosnia And Herzegovina; 2021 [cited 2022 Aug 9].

410. Federation of Bosnia and Herzegovina. O Proglašenju Zakona O Zaštiti Stanovništva Od Zaraznih Bolesti [Law On The Protection Of The Population Against Infectious Diseases]. No.29/05. 2005 May.

411. Republic of Botswana. Ministry of Health [Internet]. 2023 [cited 2023 Jan 16].

412. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Republic of Botswana, Mission report 4-8 December 2017. No. WHO/WHE/CPI/REP/2018.18. Geneva: World Health Organization; 2018.

413. Ministry of Health. Botswana Integrated Disease Surveillance & Response. Botswana: Republic of Botswana; 2019 Jul.

414. Government of Botswana. Public Health Act. No. 63:01**.** Botswana: Botswana; 2007.

415. Ministry of Health. Integrated Health Service Plan: A Strategy for Changing the Health Sector For Healthy Botswana 2010-2020. Botswana: Government of Botswana; 2010 Nov.

416. Federative Republic of Brazil. Ministry of Health [Internet]. 2023 [cited 2023 Jan 16].

417. Ministry of Health Brazil. Epidemiological Bulletin December 2022. No.53-48. 2022 Dec.

418. Ministry of Health Brazil. National List of Compulsory Notification of Diseases, Injuries and Public Health Events [Internet]. 2022 [cited 2023 Jan 17].

419. Ministry of Health Brazil. Plano de Operação do Ponto Focal Nacional para o Regulamento Sanitário Internacional [Operation Plan of the National Focal Point for the International Health Regulations]. Brazil: Departamento de Vigilância das Doenças Transmissíveis; 2016.

420. Government of Brazil. Plano de Fortalecimento e Ampliação da Rede Nacional de Vigilância Epidemiológica Hospitalar [Plan for Strengthening and Expansion of the National Hospital Epidemiological Surveillance Network]. Brazil: Ministry of Health; 2022 May.

421. Federative Republic of Brazil. National List of Compulsory Notification of Diseases [Internet]. Ministry of Health; 2014 Jun [cited 2023 Jan 16].

422. Ministry of Health Brazil. Sistema de Informação de Agravos de Notifi cação [Information System of Notifiable Diseases] [Internet]. Brazil: Departamento de Vigilância Epidemiológica; 2006 [cited 2022 Aug 12].

423. Federative Republic of Brazil. Ficha De Investigação De Surto [Outbreak Investigation Form]. Brazil: Ministry of Health; 2006 May.

424. Federative Republic of Brazil. Surto Instruções Para Preenchimento Ficha De Investigação [Outbreak Instructions for Completing the Investigation Form]. Brazil: Ministry of Health; 2006 May.

425. Ministry of Health Brunei. Communicable Diseases Surveillance [Internet]. 2022 [cited 2022 Jul 27].

426. Brunei. Infectious Diseases Act. No.S 34/03. Brunei; 2010.

427. World Health Organization. Joint external evaluation of IHR core capacities of Brunei Darussalam, Mission report: 28 October - 1 November 2019. No.9789240006973. Geneva: World Health Organization; 2020.

428. Ministry of Health Brunei Darussalam. Strategic Plan 2019-2023. Department of Policy & Planning; 2019.

429. Republic of Bulgaria. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

430. Republic of Bulgaria. Epidemic situation 19.12.2022 - 25.12.2022 [Internet]. Ministry of Health. 2022 [cited 2023 Jan 17].

431. Republic of Bulgaria. ЗАКОН ЗА ЗДРАВЕТО [Health Law]. Bulgaria; 2005 Jan.

432. Mira Kojouharova. Overview Of Communicable Disease Surveillance System In Bulgaria. Bulgaria: National Centre Of Infectious And Parasitic Diseases, Bulgaria; 2011 Mar.

433. Ministère de la Santé et de l'Hygiène Publique. Ministry of Health and Public Hygiene [Internet]. Burkina Faso. 2022 [cited 2022 Jul 27].

434. World Health Organization. Évaluation externe conjointe des principales capacités RSI du Burkina Faso, rapport de mission: 4-8 décembre 2017 [Joint external evaluation of IHR core capacities of Burkina Faso, Mission report: 4-8 December 2017]. No. WHO/WHE/CPI/REP/2018.12. Geneva: World Health Organization; 2018.

435. Ministry of Health Burkina Faso. Guide Technique Pour La Surveillance Integree De La Maladie Et La Riposte Au Burkina Faso [technical Guide For Monitoring Integrated Disease And Response In Burkina Faso]. Burkina Faso; 2012.

436. Allison M. Connolly, Issaka Sawadogo, Eric Geers, M. Yolande B. Eugene. Implementing Event-Based Surveillance in Burkina Faso Using the "One Health" Approach. Burkina Faso: Measure Evaluation, University of North Carolina at Chapel Hill; 2019 Sep.

437. Republic of Burundi. Ministry of Public Health [Internet]. 2023 [cited 2023 Jan 17].

438. Republic of Burundi. National Institute of Public Health [Internet]. 2023 [cited 2023 Jan 17].

439. Republic of Burundi. Portant Code de la Santé Publique [Public Health Code]. Burundi; 1982.

440. Republic of Cabo Verde. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

441. World Health Organization. Joint External Evaluation of IHR Core Capacities of Cabo Verde, Mission report 4-8 November 2019. Geneva: World Health Organization; 2020.

442. Republic of Cabo Verde. Relatório Estatístico 2018 [Statistical Report 2018] [Internet]. Cabo Verde: Ministry of Health; 2019 [cited 2022 Jul 28].

443. Republic of Cabo Verde. National Institute of Public Health [Internet]. 2023 [cited 2023 Jan 17].

444. Kingdom of Cambodia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

445. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Cambodia, Mission report: 26 August - 2 September 2016. No. WHO/WHE/CPI/2017.11. Geneva: World Health Organization; 2017.

446. Ministry of Health Cambodia. Weekly Surveillance Report Week 51-2022 [Internet]. CamEWARN. 2022 [cited 2023 Jan 17].

447. Ministry of Public Health Cameroon. National Observatory of Public Health [Internet]. 2022 [cited 2022 Jul 28].

448. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Republic of Cameroon, Mission report 25-29 September 2017. No. WHO/WHE/CPI/REP/2017.60. Geneva: World Health Organization; 2017.

449. Ministry of Public Health. Tracking 100 Core Health Indicators in Cameroon. Cameroon: World Health Organization; 2019.

450. Government of Canada. Public Health Agency of Canada [Internet]. 2023 [cited 2023 Jan 17].

451. World Health Organization. Joint external evaluation of IHR core capacities of Canada, Mission report: 11-20 June 2018. No. WHO/WHE/CPI/2019.62. Geneva: World Health Organization; 2019..

452. Public Health Agency of Canada. Nationally notifiable diseases [Internet]. 2021 [cited 2022 Jul 22].

453. Government of Canada. Notifiable Disease Charts [Internet]. Public Health Agency of Canada. 2020 [cited 2023 Jan 17].

454. Government of Canada. Public Health Agency of Canada Act. No.S.C 2006, c5. 2015 Feb.

455. Canadian Public Health Laboratory Network. Strategic Plan 2016-2020. Canada; 2016.

456. Central African Republic. Ministry of Health and Population. 2023 [cited 2023 Jan 17].

457. World Health Organization. Joint External Evaluation of IHR Core Capacities of Central African Republic, Mission report 26-30 November 2018. No. WHO/WHE/CPI/2019.30. Geneva: World Health Organization; 2019.

458. Republic of Chad. Ministry of Health and National Solidarity [Internet]. 2023 [cited 2023 Jan 17].

459. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Republic of Chad, Mission report 7-11 August 2017. No. WHO/WHE/CPI/REP/2018.3. Geneva: World Health Organization; 2018.

460. Republic of Chad. Politique Nationale De Santé 2016-2030 [National Health Policy 2016-2030]. Chad;2016.

461. Republic of Chile. Institute of Public Health [Internet]. Ministry of Health. 2022 [cited 2022 Jul 27].

462. Ministry of Health Chile. Bulletin Public Health Events July 2022 [Internet]. Chile: Government of Chile; 2022 Aug [cited 2023 Jan 17].

463. People's Republic of China. Chinese Center for Disease Control and Prevention [Internet]. 2023 [cited 2023 Jan 17].

464. People's Republic of China. 国家突发公共卫生事件应急预案 [National Emergency Response Plan for Public Health Emergencies] [Internet]. China; 2006 Feb [cited 2022 Jul 28].

465. Wang L, Ren X, Cowling BJ, Zeng L, Geng M, Wu P, et al. Systematic review: national

notifiable infectious disease surveillance system in China. Online J Public Health Inform. 2019;11(1):e414.

466. Chinese Center for Disease Control and Prevention. Reported Cases and Deaths of National Notifiable Infectious Diseases - China, November 2022 [Internet]. 2022 [cited 2023 Jan 17].

467. Government of Colombia. National Institute of Health [Internet]. 2023 [cited 2023 Jan 17].

468. Ministry of Health Colombia. Weekly Epidemiological Bulletin - 52 [Internet]. 2022 [cited 2023 Jan 17].

469. National Institute of Health Colombia. Lineamientos Nacionales Para La Vigilancia 2022 [National Guidelines For Surveillance 2022]. Colombia; 2021.

470. Republic of Colombia. Regulations: laws. Ministry of Health and Social Protection. 2023.

471. National Institute of Health Colombia. Protocolo de Vigilancia de Infección Respiratoria Aguda [Surveillance Protocol for Acute Respiratory infections]. Colombia; 2022.

472. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Union of the Comoros, Mission report 20-25 August 2017. No. WHO/WHE/CPI/REP/2018.6. Geneva: World Health Organization; 2018.

473. Solet JL, Filleul L, Gedeon J, Polycarpe D, Nundlall TR, Randrianarivo-Solofoniaina AE. SEGA: the Indian Ocean epidemic surveillance and alert management network. Bulletin épidémiologique hebdomadaire. 2014;(7):130–5.

474. Union of Comoros. Code de la santé publique et de l'action sociale [Public Health and Action Code]. No.95-013. Comoros; 1995 Jun.

475. Union of the Comoros. Comoros Ministry of Health [Internet]. 2022 [cited 2022 Jul 29].

476. Republic of the Congo. Ministry of Health [Internet]. 2022 [cited 2022 Jul 29].

477. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Republic of Congo. No. WHO/WHE/CPI/2019.60. Geneva: World Health Organization; 2019 Jun.

478. Ministry of Health. Bulletin épidémiologique de surveillance integrée des maladies et riposte de la République du Congo [Epidemiological bulletin of integrated disease surveillance and response of the Republic of Congo] [Internet]. Congo (Brazzaville): Republic of Congo; 2020 May [cited 2022 Jul 29]. Report No.52.

479. Democratic Republic of the Congo. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

480. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Democratic Republic of Congo, Mission report, March 12-16, 2018. No. WHO/WHE/CPI/2018.28. Geneva: World Health Organization; 2018.

481. Democratic Republic of the Congo. Institut National de la Recherche Biomédicale [National Institute of Biomedical Research] [Internet]. 2023 [cited 2023 Jan 17].

482. Mandja BAM, Bompangue D, Handschumacher P, Gonzalez JP, Salem G, Muyembe JJ, et al. The score of integrated disease surveillance and response adequacy (SIA): a pragmatic score for comparing weekly reported diseases based on a systematic review. BMC Public Health. 2019;19(1):1–14.

483. Democratic Republic of the Congo. Plan National de Développement Sanitaire recadré pour la période 2019-2022 [National Health Development Plan 2019-2022]. 2018.

484. Cook Islands. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

485. Cook Islands. Public Health Act. Cook Islands; 2004.

486. Ministry of Health Cook Islands. Public Health (Notifiable Conditions) Order 2007. Cook Islands: Government of the Cook Islands; 2007 Feb.

487. Costa Rica. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

488. Costa Rican Institute for Research and Teaching in Nutrition and Health. Eventos bajo vigilancia [Events under surveillance] [Internet]. 2022 [cited 2022 Jul 28].

489. Ministry of Health of Costa Rica. Epidemiological Bulletin No 50 of 2022 [Internet]. 2022 [cited 2023 Jan 17].

490. Republic of Costa Rica. General Health Law No 5395. 1973.

491. Republic of Côte d'Ivoire. Ministry of Health, Public Hygiene and Universal Health [Internet]. 2023 [cited 2023 Jan 17].

492. Côte d'Ivoire. National Institute of Public Hygiene [Internet]. 2022 [cited 2022 Jul 30].

493. Côte d'Ivoire. Creating the National Public Health Laboratory Decree No. 91-654. 1991.

494. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Côte d'Ivoire, Mission report 5-9 December 2016. No. WHO/WHE/CPI/2017.20. Geneva: World Health Organization; 2017.

495. Japan International Cooperation Agency (JICA). Republic of Côte d'Ivoire [Internet]. République de Côte d'Ivoire; 2022 Mar [cited 2023 Jan 16].

496. Republic of Croatia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

497. Republic of Croatia. On The Protection Of The Population Against Infectious Diseases. Croatia; 2007.

498. Croatian Institute Of Public Health. Communicable Diseases In Croatia 2016 [Internet]. Croatia: Ministry of Health Croatia; 2016 [cited 2023 Jan 5].

499. Republic of Cuba. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

500. Republic of Cuba. Statistical Yearbook of Health 2020 [Internet]. 2021 [cited 2023 Jan 17].

501. Republic of Cuba. Public Health Law No 41. 1983.

502. Wenham C, Kittelsen SK. Cuba y seguridad sanitaria mundial: Cuba's role in global health security. BMJ global health. 2020;5(5):e002227.

503. Republic of Cyprus. Ministry of Health [Internet]. 2022 [cited 2022 Aug 2].

504. Republic of Cyprus. Quarantine Law. No.145. Cyprus; 1953 Jan.

505. Ministry of Health Cyprus. Strategic Plan 2022-2024 [Internet]. 2021 [cited 2022 Aug 2].

506. Czech Republic. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

507. Ministry of Health Czech Republic. Pandemický plán České republiky [Pandemic plan of the Czech Republic]. Czech Republic; 2012 Jul.

508. Czech Republic. Cases of selected infectious diseases in the Czech Republic, December 2022 [Internet]. 2023 [cited 2023 Jan 17].

509. Czech Republic. Protection of Public Health Act No 258. 2001.

510. Kingdom of Denmark. Ministry of the Interior and Health [Internet]. 2023 [cited 2023 Jan 17].

511. Statens Serum Institut Denmark. Monitoring in numbers, graphs and maps [Internet]. 2023 [cited 2023 Jan 17].

512. Kingdom of Denmark. Bekendtgørelse om alment farlige sygdomme [Proclamation on generally dangerous diseases]. No. 304. Denmark; 2021 Feb.

513. Kingdom of Denmark. Bekendtgørelse om lægers anmeldelse af smitsomme sygdomme [Order on doctors' notification of infectious diseases]. Denmark; 2000 Apr.

514. Republic of Djibouti. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

515. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la République de Djibouti Rapport de mission: 1-5 juillet 2018 [Joint External Evaluation of IHR Core Capacities of the Republic of Djibouti, Mission report: July 1-5, 2018]. No. WHO/WHE/CPI/REP/2018.29. Geneva: World Health Organization; 2018.

516. Republic of Djibouti. Law No. 118 Relating to the powers and organization of the Ministry of Health. 1992.

517. Commonwealth of Dominica. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

518. Commonwealth of Dominica. Environmental Health Services (Communicable and Notifiable Diseases) Regulations. Dominica; 2003.

519. Commonwealth of Dominica. Environmental Health Services (Communicable and Notifiable Diseases) (Amendment) Regulations. 2020.

520. Dominican Republic. Ministry of Health and Social Assistance [Internet]. 2022 [cited 2022 Aug 7].

521. Ministry of Public Health Dominican Republic. Guía para la elaboración del Plan Hospitalario para Respuesta a Emergencias y Desastres [Hospital Plan for Response to Emergencies and Disasters]. Santo Domingo, República Dominicana: National Directorate of Emergencies and Disasters; 2013.

522. Ministry of Health Dominican Republic. Epidemiological Bulletin Week 40 [Internet]. 2022 [cited 2022 Dec 30].

523. Dominican Republic. Mandatory Report of Prioritized Diseases and Events [Internet]. Dominican Republic; 2020 Feb [cited 2023 Jan 5]. Report No.4.

524. Republic of Ecuador. Ministry of Public Health [Internet]. 2023 [cited 2023 Jan 17].

525. Ministry of Public Health Republic of Ecuador. National Directorate of Epidemiological Surveillance [Internet]. 2022 [cited 2022 Aug 3].

526. Ministry of Public Health Ecuador. Weekly Epidemiological Report 40 [Internet]. 2022 [cited 2023 Jan 2].

527. Ministry of Public Health Republic of Ecuador. Sistema Integrado De Vigilancia Epidemiológica Manual De Procedimientos [Integrated Epidemiological Surveillance System Procedures Manual] [Internet]. 2013 [cited 2023 Jan 17].

528. Republic of Egypt. Ministry of Health and Population [Internet]. 2023 [cited 2023 Jan 17].

529. Abdel-Razik MSM, Rizk HII, Hassan MHM. Surveillance of communicable diseases for decision-making in Egypt: 2006–2013. East Mediterr Health J. 2017;23(6):395.

530. El Sood HA, Kamer SAA, Kamel R, Magdy H, Osman FS, Fahim M, et al. The impact of implementing the egypt pandemic preparedness plan for acute respiratory infections in combating the early stage of the COVID-19 pandemic, February-July 2020. JMIR Public Health and Surveillance. 2021;7(5):e27412.

531. El Salvador. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

532. Ministry of Health El Salvador. Epidemiological Bulletin Week 52 [Internet]. 2023 [cited 2023 Jan 17].

533. Republic of El Salvador. Health Code Decree No 955. 1988.

534. El Salvador. Laboratorio Nacional de Referencia [National Reference Laboratory] [Internet]. 2022 [cited 2022 Aug 3].

535. Ministry of Health El Salvador. Lineamientos técnicos del Sistema Nacional de Vigilancia Epidemiológica en El Salvador [Technical guidelines of the National System of Epidemiological Surveillance in El Salvador]. El Salvador; 2019 Aug.

536. Equatorial Guinea. Ministry of Health and Social Welfare [Internet]. 2023 [cited 2023 Jan 17].

537. Republic of Equatorial Guinea. Estratégico De Fortalecimiento Del Sistema Nacional De Información Sanitaria [Strategic Strengthening Of The National Health Information System]. Equatorial Guinea: Ministry of Health and Social Welfare; 2019 Feb.

538. World Health Organization. Joint External Evaluation of IHR Core Capacities of the State of Eritrea, Mission report October 2016. No. WHO/WHE/CPI/2017.12. Geneva: World Health Organization; 2017.

539. Eritrea. Ministry of Information [Internet]. 2023 [cited 2023 Jan 17].

540. Republic of Estonia. Health Board [Internet]. 2023 [cited 2023 Jan 17].

541. Republic of Estonia. Prevention and Control of Infectious Diseases Act. Estonia: Parliament; 2003 Feb.

542. Health Board Republic of Estonia. Nakkushaigustesse haigestumise statistilised andmed [Statistical data on the incidence of infectious diseases] [Internet]. 2023 [cited 2023 Jan 17].

543. Republic of Estonia. The procedure for transmitting information about the presence of infectious diseases [Internet]. 2020 [cited 2023 Jan 17].

544. Kingdom of Eswatini. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

545. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Eswatini, Mission report 9-13 April 2018. No. WHO/WHE/CPI/2018.27. Geneva: World Health Organization; 2018.

546. Kingdom of Eswatini. Public Health Act. Eswatini: Kingdom of Eswatini; 1969 Aug [cited 2023 Jan 5].

547. Ethiopia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

548. World Health Organization. Joint external evaluation of IHR core capacities of the Federal Democratic Republic of Ethiopia, Mission report March 2016. No. WHO/HSE/GCR/2016.24. Geneva: World Health Organization; 2017.

549. The Ethiopian Public Health Institute. Public Health Emergency [Internet]. 2021 [cited 2022 Aug 4].

550. Ethiopian Public Health Institute. Weekly Epidemiological Bulletin - Week 29 [Internet]. Ethiopia: Center for Public Health Emergency Management; 2022 Jul [cited 2023 Jan 5].

551. Ethiopian Public Health Institute. Community And Event-based Surveillance [Internet]. Ethiopia: Center For Public Health Emergency Management; 2021 Sep [cited 2023 Jan 16].

552. Fiji. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

553. Ministry of Health and Medical Services Fiji. Communicable Disease Surveillance and Outbreak Response Guidelines. Fiji: Republic of Fiji; 2016 Dec.

554. Republic of Fiji. Public Health Act 1935; Amended 2021. Fiji; 2021 Jul. Report No.6.

555. Republic of Finland. Ministry of Social Affairs and Health [Internet]. 2023 [cited 2023 Jan 17].

556. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Finland, Mission report: March 2017. No. WHO/WHE/CPI/2017.24. Geneva: World Health Organization; 2017.

557. Ministry of Social Affairs and Health Finland. Notifiable diseases and microbes [Internet]. 2022 [cited 2022 Aug 5].

558. Finnish Institute for Health and Welfare. Infectious diseases in Finland 2021 [Internet]. 2022 [cited 2023 Jan 17].

559. Republic of Finland. Tartuntatautilaki [Infectious Diseases Act]. No.1227/2016. Finland; 2016 Dec.

560. Republic of France. Ministry of Health and Prevention [Internet]. 2023 [cited 2023 Jan 17].

561. Public Health France. Notifiable Disease Surveillance System [Internet]. 2003 [cited 2022 Aug 5].

562. Public Health France. Liste des maladies à déclaration obligatoire [List of Notifiable Diseases] [Internet]. 2023 Jan 2 [cited 2023 Jan 17].

563. Public Health France. Bulletin épidémiologique hebdomadaire [Weekly epidemiological bulletin] [Internet]. 2023 [cited 2023 Jan 17].

564. Public Health France. Géodes: Géo données en santé publique [Geodata in public health] [Internet]. 2023 [cited 2023 Jan 27].

565. Gabon. Ministry of Health and Social Affairs [Internet]. 2023 [cited 2023 Jan 17].

566. World Health Organization. Évaluation externe conjointe des principales capacités RSI en République Gabonaise Rapport de mission, 1-5 Julliet 2019 [Joint external evaluation of IHR core capacities in the Gabonese Republic, Mission report, 1-5 July 2019]. No. WHO/WHE/CPI/2019.18. Geneva: World Health Organization; 2019.

567. Ministry of Health Gabon. National Plan for Health Development 2017-2021. Gabon; 2017.

568. Gambia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

569. Gambia. Public Health Act No 1. Gambia;1989.

570. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of the Gambia, Mission report: 24-29 September 2017. No.

WHO/WHE/CPI/REP/2017.61. Geneva: World Health Organization; 2017.

571. Georgia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

572. World Health Organization. Joint external evaluation of IHR core capacities of Georgia, Mission report: 10-14 June, 2019. No. WHO/WHE/CPI/2019.13. Geneva: World Health Organization; 2019.

573. National Center for Disease Control and Public Health Georgia. Electronic Integrated Disease Surveillance System [Internet]. 2018 [cited 2022 Aug 6].

574. Georgia. Public Health Law No 5069; Amendments 15 Dec 2022. Georgia; 2007.

575. Global Health Security Agenda. Global Health Security Agenda Pilot Assessment Of Georgia. Georgia; 2015 Jan.

576. National Public Health Center for Disease Control Georgia. Health protection statistical reference 2018 [Internet]. 2019 [cited 2022 Aug 7].

577. Federal Republic of Germany. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

578. Ministry of Health Germany. Nationaler Pandemieplan [National Pandemic Plan]. Germany: Robert Koch Institute; 2017 Mar [cited 2023 Jan 5].

579. Robert Koch Institute Germany. Epidemiology Bulletin - 50 [Internet]. 2022 [cited 2023 Jan 17].

580. Robert Koch Institute Germany. International Health Regulations [Internet]. 2022 [cited 2023 Jan 17].

581. Federal Republic of Germany. Infection Protection Act; last amended December 20, 2022. Germany; 2022.

582. Ghana. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

583. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Ghana, Mission report 6-10 February 2017. No. WHO/WHE/CPI/2017.26. Geneva: World Health Organization; 2017.

584. Ministry of Health. National Guidelines for Lab Testing and Reporting on Resp Infectious Diseases in Health Facilities in Ghana. Ghana: Republic of Ghana; 2020.

585. Ministry of Health. Communicable Diseases Analysis Book A module of the Technical Guidelines for Integrated Disease Surveillance and Response in Ghana. Ghana: Republic of Ghana; 2005.

586. Ghana Ministry of Health. Summary Of Priority Diseases And Events - Week 24, 2021 [Internet]. Ghana; 2021 [cited 2023 Jan 5].

587. Republic of Ghana. Public Health Act No 851. Ghana; 2012.

588. Greece. Ministry Of Health And Social Solidarity [Internet]. 2023 [cited 2023 Jan 17].

589. Ministry of Health Greece. Reformulation of the list of mandatory notifiable diseases No 16711/2022. Greece; 2022.

590. Ministry of Health Greece. Directorate Of Epidemiology Surveillance And Intervention For Infectious Diseases Surveillance Data, October 2022 [Internet]. 2022 [cited 2023 Jan 17].

591. Ministry of Health & Social Solidarity Greece. Εθνικό Σχέδιο Δράσης για την Πρόληψη των Μεταδοτικών Νοσημάτων 2008 - 2012 [National Action Plan for preventing Communicable Diseases 2008 - 2012]. Greece; 2008.

592. Grenada. Ministry of Health [Internet]. 2022 [cited 2022 Aug 8].

593. Grenada. Public Health Act No 263. Grenada; 1981.

594. Peters S. Notifiable Disease Reporting among Physicians Practicing in Grenada. Online J Public Health Inform. 2017;9(1).

595. Guatemala. Ministry of Public Health and Social Assistance [Internet]. 2022 [cited 2022 Aug 8].

596. Ministry of Public Health and Social Assistance. Monitoring Protocol Of Epidemiological Surveillance [Internet]. Guatemala: Guatemala; 2018 [cited 2023 Jan 16].

597. Ministry of Public Health and Social Assistance. Protocolo del Sistema Nacional de Vigilancia Epidemiológica [Protocol of the National System of Epidemiological Surveillance]. Guatemala: Guatemala; 2018.

598. Guatemala. Health Code Decree No 90-97. 1997 [cited 2023 Jan 17].

599. Ministry of Public Health and Social Assistance. Epidemiology Week 34 Guatemala [Internet]. 2022 [cited 2023 Jan 17].

600. Guinea. Ministry of Health [Internet]. 2023 [cited 2023 Jan 17].

601. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la Répubiique de Guinée Rapport de mission, 23-28 avril 2017 [Joint external evaluation of the RSI core capacities of the Republic of Guinea, Mission report, April 23-28, 2017]. Geneva: World Health Organization; 2017. WHO/WHE/CPI/REP/2017.40.

602. National Health Security Agency Guinea. Les Maladies sous surveillance [Diseases under surveillance] [Internet]. 2022 [cited 2022 Aug 11].

603. Republic of Guinea. Public Health Code. Guinea; 1997.

604. Ministry of Health. Weekly Epidemiological Bulletin Guinea [Internet]. Guinea: Republic of Guinea; 2019 Sep [cited 2023 Jan 5].

605. World Health Organization. Joint external evaluation of IHR core capacities of the

Republic of Guinea-Bissau, Mission report 8-12 July 2019. No. WHO/WHE/CPI/2019.17. Geneva: World Health Organization; 2020.

606. Guinea-Bissau. Infracções antieconómicas e contra a saúde pública [Anti-economic and public health offenses]. Decree No 20/77. Guinea-Bissau; 1977.

607. Guerreiro CS, Ferrinho P, Hartz Z. Health evaluation in the Republic of Guinea-Bissau: A meta-evaluation of the National Health Development Plan. Saúde em Debate. 2018;42:549–65.

608. Guinea-Bissau. Ministry of Public Health [Internet]. 2023 [cited 2023 Jan 18].

609. Guyana. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

610. Republic of Guyana. Public Health Act No 15. Parliament of the Co-operative Republic of Guyana. 1934.

611. Ministry of Health. Guyana COVID-19 Emergency Response Project [Internet]. Guyana: Environmental and Social Management Framework; 2022 Mar [cited 2023 Jan 18].

612. Haiti. Ministry of Public Health and Population [Internet]. 2023 [cited 2023 Jan 18].

613. Ministry of Public Health and Population Haiti. Report of Diseases / Phenomena Under Epidemiological Surveillance - Week 7 [Internet]. 2020 [cited 2022 Aug 6].

614. National Public Health Laboratory Haiti. Role Of The Laboratory Network In The Management Of Epidemics And Health Emergencies [Internet]. Haiti: Ministry of Public Health and Population; 2020 Sep [cited 2022 Aug 6].

615. Republic of Haiti. Decree on the organization and functioning of the Ministry of Public Health and Population. No 88. Haiti; 2020 May 21.

616. Juin S, Schaad N, Lafontant D, Joseph GA, Barzilay E, Boncy J, et al. Strengthening national disease surveillance and response—Haiti, 2010–2015. Am J Trop Med Hyg. 2017;97(4S):12.

617. Republic of Honduras. Ministry of Health. 2022 [cited 2022 Aug 7].

618. Republic of Honduras. Decree 65/91 - Código de Salud [Health Code]. 1991.

619. Health Secretary Honduras. Plan Antipandemico Influenza [Influenza Pandemic Plan]. Report No.PCM-026-2018. Honduras: Republic of Honduras; 2018.

620. Hungary. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

621. Hungary. Rendelet a fertőző betegségek és a járványok megelőzése érdekében szükséges járványügyi intézkedésekről [Decree on the necessary epidemiological measures to prevent infectious diseases and epidemics]. Hungary; 1998.

622. Hungary Ministry of Human Capacities. Epidemiological Weekly Situational Report - Week 42 [Internet]. Hungary; 23-Oct-2022 [cited 2023 Jan 5].

623. Ministry of Health Hungary. Jelentendő betegségek [Reportable diseases] [Internet]. 2014 [cited 2022 Aug 7].

624. Iceland. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

625. Directorate of Health Iceland. Transmission and prevention [Internet]. 2022 [cited 2022 Aug 7].

626. Republic of Iceland. Sóttvarnalög [Epidemic Prevention Act]. No. 13. Iceland; 2022 Sep.

627. Directorate of Health Iceland. Um skráningarskyldu [Obligation to register] [Internet]. Iceland; 2019 Feb [cited 2023 Jan 5].

628. Directorate of Health Iceland. Farsóttasýrsla 2020 [Epidemic report 2020] [Internet]. Iceland; 2022 May [cited 2023 Jan 5].

629. Directorate of Health Iceland. Leiðbeiningar til lækna varðandi tilkynningar [Instructions for doctors regarding notifications] [Internet]. Iceland; 2020 Oct [cited 2023 Jan 5]. Report No.221/2012.

630. Directorate of Health Iceland. Óvæntir atburðir sem ógnað geta heilsu manna [Unexpected events that can threaten human health] [Internet]. Iceland; 2022 [cited 2023 Jan 18].

631. Republic of India. Ministry of Health and Family Welfare [Internet]. 2023 [cited 2023 Jan 18].

632. Ministry of Health and Family Welfare India. Weekly Reporting Format [Internet]. India: Integrated Disease Surveillance Programme; 2022 [cited 2022 Aug 8].

633. Ministry of Law and Justice India. The Epidemic Diseases Act No 3; amended 14 Jan 2019. India; 1897 Feb.

634. Integrated Disease Surveillance Program India. Weekly Outbreak Report - 45th Week 2022 [Internet]. 2022 [cited 2023 Jan 18].

635. Integrated Disease Surveillance Project India. Reporting Format for Syndromic Surveillance [Internet]. India; 2022 [cited 2022 Aug 8].

636. Ministry of Health Republic of Indonesia. Emerging Infectious Diseases [Internet]. 2022 [cited 2022 Aug 8].

637. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Indonesia, Mission report: 20-24 November 2017. Geneva: World Health Organization; 2017. WHO/WHE/CPI/REP/2018.9.

638. Ministry of Health Indonesia. Development of the Situation of Emerging Infectious Diseases 52nd Epidemiology Week in 2022 [Internet]. 2023 [cited 2023 Jan 18].

639. Republic of Indonesia. Sistem Kesehatan Nasional [National Health System] Decree 72/2012. Indonesia; 2012.

640. Republic of Indonesia. Communicable Disease Management No 1755 [Internet]. Regulations of the Ministry of Health of the Republic of Indonesia. 2014 [cited 2023 Jan 18].

641. Ministry of Health Republic of Indonesia. Sistem Kewaspadaan Dini dan Respon [Early Alertness and Response System] [Internet]. 2021 [cited 2022 Aug 8].

642. Islamic Republic of Iran. Ministry of Health and Medical Education [Internet]. 2023 [cited 2023 Jan 18].

643. Islamic Republic of Iran. Law on the prevention of communicable diseases. Iran; 2019.

644. Moradi G, Asadi H, Gouya MM, Nabavi M, Norouzinejad A, Karimi M, Mohamadi-Bolbanabad A. The Communicable Diseases Surveillance System in Iran: Challenges and Opportunities. Arch Iran Med. 2019 Jul 1;22(7):361-368.

645. Nader F, Askarian M. How do Iranian physicians report notifiable diseases? The first report from Iran. Am J Infect Control. 2009 Aug;37(6):500-4.

646. Republic of Iraq. Ministry of Health [Internet]. 2022 [cited 2022 Aug 8].

647. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Iraq, Mission report: 12-17 March 2019. No. WHO/WHE/CPI/2019.61. Geneva: World Health Organization; 2019.

648. Republic of Iraq. Public Health Law No 89. Iraq; 1981.

649. Republic of Iraq Ministry of Health. Weekly Report of Reported Communicable Diseases all over Iraq 2019 - Week 13 [Internet]. 2019 [cited 2022 Aug 8].

650. Hamalaw SA, Bayati AH, Babakir-Mina M. An Assessment of Timeliness and Quality of Communicable Disease Surveillance System in the Kurdistan Region of Iraq. Inquiry. 2021 Jan-Dec;58:469580211056045.

651. Ireland. Department of Health [Internet]. 2023 [cited 2023 Jan 18].

652. Health Protection Surveillance Centre Ireland. Notifiable Diseases and their respective causative pathogens [Internet]. Ireland; 2022 May [cited 2023 Jan 5].

653. Ministry of Health Ireland. Infectious Diseases (Amendment) Regulations [Internet]. Ireland; 2022 May [cited 2023 Jan 5]. No.258/2022.

654. Health Service Executive Ireland. Infectious Disease Outbreak Reporting Form. Ireland; 2014 Jan [cited 2023 Jan 5].

655. Health Protection Surveillance Centre Ireland. Weekly Outbreak Report - Week 52, 2022 [Internet]. 2023 [cited 2023 Jan 18].

656. State of Israel. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

657. Ministry of Health Israel. Public Health Ordinance, 1940. Israel; 2018 Jul.

658. Ministry of Health Israel. תקנות בריאות העם [Public Health Regulations]. Israel; 2021.

659. Ministry of Health Israel. Epidemiological report Weekly report number 49 [Internet]. 2022 [cited 2023 Jan 18].

660. State of Israel. Notifiable infectious diseases in Israel: 60 years of monitoring 1951-2010 [Internet]. 2012 [cited 2022 Aug 8].

661. Republic of Italy. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

662. Ministry of Health Italy. Piano strategico-operativo nazionale di preparazione e risposta a una pandemia influenzale (PanFlu) 2021-2023 [National strategic-operational plan of preparation and response to a flu pandemic (PanFlu) 2021-2023] [Internet]. Italy; 2021 Jan [cited 2023 Jan 5].

663. Republic of Italy. Ministry of Health Decree: Information system of infectious and diffusive diseases [Internet]. 1990 [cited 2023 Jan 18].

664. Ministry of Health Italy. Modalità di segnalazione delle malattie [Methods of reporting diseases] [Internet]. Italy; 2022 [cited 2023 Jan 5].

665. Higher Institute of Health Italy. Infectious diseases [Internet]. EpiCentro - Epidemiology for public health. 2023 [cited 2023 Jan 18].

666. Italian Higher Institute of Health. Surveillances and registers [Internet]. EpiCentro - Epidemiology for public health. 2023 [cited 2023 Jan 19].

667. Jamaica. Ministry of Health and Wellness [Internet]. 2023 [cited 2023 Jan 18].

668. Ministry of Health and Wellness Jamaica. Weekly Epidemiology Bulletin - Week 51 [Internet]. 2023 [cited 2023 Jan 18].

669. Jamaica. Public Health Act; Last Amendment: January 1, 1996. Jamaica; 1985 Feb.

670. Japan. Ministry of Health, Labour and Welfare [Internet]. 2023 [cited 2023 Jan 18].

671. World Health Organization. Joint external evaluation of IHR core capacities of Japan, Mission report: 26 February - 2 March 2018. Geneva: World Health Organization; 2018. WHO/WHE/CPI/REP/2018.23.

672. National Institute of Infectious Diseases Japan. Laboratory manuals for pathogen detection. Japan; 2020 Feb.

673. Japan. Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases; Amendment 2014. Japan; 1998 Oct.

674. National Institute of Infectious Diseases Japan. IDWR Flash Data Week 52, 2022 [Internet]. 2023 [cited 2023 Jan 18].

675. Infectious Disease Surveillance Centre Japan. Infectious Disease Surveillance System in Japan [Internet]. National Institute of Infectious Diseases. 2018 [cited 2022 Aug 9].

676. Kingdom of Jordan. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

677. World Health Organization. Joint external evaluation of IHR core capacities of the Hashemite Kingdom of Jordan, Mission report: 28 August -1 September 2016. No. WHO/WHE/CPI/2017.01. Geneva: World Health Organization; 2017.

678. Kingdom of Jordan. Public Health Law No 47; amended 4 Apr 2017. 2008.

679. Ministry of Health. Jordan Public Health Surveillance [Internet]. Jordan; 2015 [cited 2022 Aug 8].

680. Abdulrahim N, Alasasfeh I, Khader YS, Iblan I. Knowledge, Awareness, and Compliance of Disease Surveillance and Notification Among Jordanian Physicians in Residency Programs. Inquiry. 2019 Jan-Dec;56:46958019856508.

681. Republic of Kazakhstan. Ministry of Healthcare [Internet]. 2022 [cited 2022 Aug 8].

682. Republic of Kazakhstan. On the health of the people and the healthcare system; amendment 12 Jan 2023. Kazakhstan; 2020.

683. Ministry of Healthcare Kazakhstan. Diseases for Weeks 51-52 of 2022 [Internet]. 2022 [cited 2023 Jan 18].

684. Bekshin Z, Esmagambetova A, Kazakov S, Burdakov A, Kobzhasarov D, Ukharov A. Modernization of Epi Surveillance in Kazakhstan: Transition to Risk Assessment and Real-Time Monitoring Based on Situational Center. Online J Public Health Inform. 2016;8(1).

685. Republic of Kenya. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

686. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Kenya, Mission report, 27 February to 3 March 2017. No. WHO/WHE/CPI/REP/2017.44. Geneva: World Health Organization; 2017.

687. Republic of Kenya. Public Health Act 242; Revised Edition 2012. Kenya; 1986.

688. Ministry of Health Kenya. Standard Case Definitions for Priority Diseases in Kenya [Internet]. Kenya; 2015 Jan [cited 2023 Jan 5].

689. Kiribati. Ministry of Health and Medical Services [Internet]. 2023 [cited 2023 Jan 18].

690. Ministry of Health Kiribati. Communicable Disease Surveillance Newsletter [Internet]. Kiribati; 2008 [cited 2022 Aug 8].

691. Republic of Kiribati. Public Health (Infectious Disease) Regulations. Kiribati; 2020 Sep.

692. State of Kuwait. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

693. World Health Organization. Joint external evaluation of IHR core capacities of the State of Kuwait, Mission report: 14-18 May 2017. No. WHO/WHE/CPI/REP/2018.11. Geneva: World Health Organization; 2017.

694. Ministry of Health of the Kyrgyz Republic. Department of Disease Prevention and State

Sanitary and Epidemiological Surveillance [Internet]. 2023 [cited 2023 Jan 18].

695. World Health Organization. Joint external evaluation of IHR core capacities of the Kyrgyz Republic, Mission report: 28 November - 2 December 2016. Geneva: World Health Organization; 2017. WHO/WHE/CPI/2017.22.

696. Kyrgyz Republic. Resolution On the Concept of Comprehensive Protection of the Population No 58 [Internet]. 2018 [cited 2022 Aug 8].

697. Kyrgyz Republic. On approval of the Agreement on Cooperation between the Member States of the Eurasian Economic Community in the field of combating infectious diseases No. 387 [Internet]. 2013 [cited 2022 Aug 8].

698. Kyrgyz Republic. Monthly Bulletin of SESiZN - 9 months of 2022 [Internet]. Department of Disease Prevention and State Sanitary and Epidemiological Surveillance. 2022 [cited 2023 Jan 18].

699. Lao People's Democratic Republic. Ministry of Health [Internet]. 2022 [cited 2022 Aug 8].

700. World Health Organization. Joint external evaluation of IHR core capacities of the Lao People's Democratic Republic, Mission report: 17-24 February 2017. No. WHO/WHE/CPI/REP/2017.35. Geneva: World Health Organization; 2017.

701. Lao People's Democratic Republic. Law On Hygiene, Disease Prevention And Health Promotion No. 08/NA. Laos; 2011 Dec.

702. Ministry of Health. Laos Health Strategy 2019 - 2023 [Internet]. Laos: United States Agency for International Development; 2019 [cited 2022 Aug 8].

703. Republic of Latvia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

704. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Latvia, Mission report: 8-12 May 2017. No. WHO/WHE/CPI/2017.27. Geneva: World Health Organization; 2017.

705. Republic of Latvia. Epidemiological Safety Law. Latvia: The Parliament of the Republic of Latvia; 2018 Apr.

706. Republic of Latvia. Infekcijas slimību reģistrācijas kārtība [Procedure for registration of infectious diseases] [Internet]. Latvia; 2022 Jun [cited 2023 Jan 5].

707. Center for Disease Prevention and Control Latvia. Infekcijas slimību reģistrācija [Registration of infectious diseases] [Internet]. 2020 [cited 2022 Aug 8].

708. Center for Disease Prevention and Control Latvia. Epidemiology Bulletin November 2022 [Internet]. 2022 [cited 2023 Jan 18].

709. Republic of Lebanon. Ministry of Public Health [Internet]. 2022 [cited 2022 Aug 9].

710. World Health Organization. Joint external evaluation of IHR core capacities of the Lebanese Republic Mission report: 25-29 July 2016. No. WHO/WHE/CPI/2017.2. World Health Organization; 2017 Feb.

711. Republic of Lebanon Ministry of Public Health. Notifiable Communicable Diseases Surveillance Guidelines. Lebanon; 2015.

712. Republic of Lebanon Ministry of Public Health. Notifiable Communicable Diseases - Communicable Diseases Surveillance Year 2022. Lebanon; 2022 Dec.

713. Republic of Lebanon. Infectious Diseases Law. Lebanon; 1957.

714. Kingdom of Lesotho. Ministry of Health [Internet]. 2022 [cited 2022 Aug 8].

715. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Lesotho, Mission report 10-14 July 2017. No. WHO/WHE/CPI/REP/2017.47. Geneva: World Health Organization; 2017.

716. Kingdom of Lesotho. The Public Health Order 1970. Lesotho; 1970.

717. Republic of Liberia. Ministry of Health [Internet]. 2022 [cited 2022 Aug 8].

718. National Health Public Institute Of Liberia. Health [Internet]. 2022 [cited 2022 Aug 8].

719. Ministry of Health. Liberia IDSR Epidemiology Bulletin. Liberia: Republic of Liberia; 2019.

720. Libya. Ministry of Health [Internet]. 2022 [cited 2022 Aug 9].

721. World Health Organization. Joint external evaluation of IHR core capacities of Libya, Mission report: 9-15 July 2018. No. WHO/WHE/CPI/2019.37. Geneva: World Health Organization; 2019.

722. Libya. Law No.106 of 1973 on Health. Libya; 2021 Feb.

723. National Center for Disease Control. Libya EWARN Bulletin week 38-2021 [Internet]. 2021 [cited 2023 Jan 18].

724. The Federal Council. Federal Office of Public Health [Internet]. 2022 [cited 2022 Aug 9].

725. World Health Organization. Joint external evaluation of IHR core capacities of the Swiss Confederation and the Principality of Liechtenstein, Mission report: 30 October - 3 November 2017. No. WHO/WHE/CPI/2018.26. Geneva: World Health Organization; 2018.

726. Federal Office of Public Health. Influenza-Pandemieplan [Influenza Pandemic Plan]. Liechtenstein; 2018 Aug.

727. Federal Office of Public Health. Reportable infectious diseases - weekly case numbers [Internet]. 2023 [cited 2023 Jan 18].

728. Federal Office of Public Health (FOPH). Verordnung des EDI über die Meldung von Beobachtungen übertragbarer Krankheiten des Menschen [EDI ordinance on the reporting of observations of communicable diseases in humans]. Liechtenstein: Government of Liechtenstein; 2022.

729. Federal Office of Public Health. Meldepflichtige übertragbare Krankheiten und Erreger Leitfaden zur Meldepflicht 2020 [Notifiable transferable diseases and pathogens: Guide to reporting requirements 2020]. Switzerland; 2020.

730. Republic of Lithuania. Ministry of Health Protection [Internet]. 2023 [cited 2023 Jan 18].

731. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Lithuania, Mission report: 19-23 November 2018. No. WHO/WHE/CPI/2019.35. Geneva: World Health Organization; 2019.

732. Ministry of Health Protection Lithuania. Sergamumas Užkrečiamosiomis Ligomis [Incidence Of Infectious Diseases] [Internet]. Lithuania: National Public Health Center under the Ministry of Health; 2022.

733. Republic of Lithuania. Law on the Prevention and Control of Communicable Diseases in Humans. Lithuania; 2011 Dec.

734. Ministry of Health Protection of the Republic of Lithuania. Dėl Privalomojo epidemiologinio registravimo objektų registravimo ir informacijos apie juos teikimo tvarkos aprašo patvirtinimo“ [On the approval of the description of the procedure for the registration of objects of mandatory epidemiological registration and the provision of information about them]. No.673. Lithuania; 2022 Jun.

735. Grand Duchy of Luxembourg. Ministry of Health [Internet]. 2023 [cited 2023 Jan 18].

736. Ministry of Health Luxembourg. Laboratoire National De Santé [National Health Laboratory] [Internet]. 2022 [cited 2022 Aug 9].

737. Grand Duchy of Luxembourg. Maladies infectieuses et agents pathogènes à déclaration obligatoire Guide de la déclaration obligatoire pour médecins et médecins-dentistes [Infectious diseases and pathogens mandatory declaration Guide to mandatory reporting for doctors and dentists]. 2019.

738. Grand Duchy of Luxembourg. Loi du 1er août 2018 sur la déclaration obligatoire de certaines maladies dans le cadre de la protection de la santé publique [Law of 1 August 2018 on the compulsory notification of certain diseases in the context of the protection of public health]. Luxembourg; 2018.

739. Grand Duchy of Luxembourg. Règlement grand-ducal du 15 février 2019 portant énumération des maladies sujettes à déclaration obligatoire [Regulation of 15 February 2019 listing the diseases subject to compulsory notification]. Luxembourg; 2019.

740. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la République de Madagascar Rapport de mission, 10-14 juillet 2017 [Joint external evaluation of the RSI core capacities of the Republic of Madagascar, Mission report, July 10-14, 2017]. No. WHO/WHE/CPI/REP/2017.66. Geneva: World Health Organization; 2018.

741. Republic of Madagascar. Law No. 2011-002 of July 15, 2011 on the Health Code. Madagascar; 2011.

742. Republic of Madagascar. Ministry of Public Health [Internet]. 2022 [cited 2023 Jan 19].

743. Republic of Madagascar. Ministry of Justice [Internet]. 2022 [cited 2023 Jan 19].

744. Republic of Malawi. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

745. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Malawi, Mission report 11-15 February 2019. No. WHO/WHE/CPI/2019.58. Geneva: World Health Organization; 2019.

746. Republic of Malawi. Public Health Act. No.34:01. Malawi; 2014 Dec.

747. Malaysia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

748. World Health Organization. Joint external evaluation of IHR core capacities of Malaysia, Mission report: 29 July-2 August 2019. No.9789240015296. Geneva: World Health Organization; 2020.

749. Ministry of Health Malaysia. Incidence Rate and Morality due to Communicable Disease, 2021 [Internet]. 2022 [cited 2023 Jan 19].

750. Ministry of Health Malaysia. Case Definitions For Infectious Diseases In Malaysia. 3rd Edition. Malaysia; 2017 Jan.

751. Malaysia. Prevention And Control Of Infectious Diseases Act; last amendment 374/2006. Malaysia; 1988 Sep.

752. Mardiah ANR, Olshansky RB, Bisri MBF. Post-Disaster Governance in Southeast Asia: Response, Recovery, and Resilient Societies. Springer Nature; 2021.

753. Ministry Of Health Malaysia. Syndromic Notification And Laboratory Investigation Manual. 2nd edition. Malaysia; 2004 Oct.

754. Ministry of Health Malaysia. Standard Operating Procedure for Potential Infectious Disease. 1st edition. Malaysia: Communicable Disease Surveillance Section; 2004.

755. Republic of Maldives. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

756. World Health Organization. Joint External Evaluation of IHR Core capacities in the Republic of Maldives, Mission report: 5-9 March, 2017. No. WHO/WHE/CPI/2017.30. Geneva: World Health Organization; 2018.

757. Republic of Maldives. Public Health Protection Act. Maldives; 2012.

758. World Health Organization. Maldives: a journey of health. Report No.9789290226116. Malaysia: Country Office for Maldives; 2017.

759. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la République du Mali Rapport de mission, 27-30 juin 2017 [Joint external evaluation of the RSI core capacities of the Republic of Mali, Mission report, June 27-30, 2017]. No. WHO/WHE/CPI/REP/2017.58. Geneva: World Health Organization; 2017.

760. Republic of Mali. Decret N°06-301/p-rm Fonctionnement De L'institut National De Recherche En Sante Publique [Operation Of The Institute National Health Research Public]. Mali; 2006.

761. Republic of Mali. Ministry of Health and Public Hygiene [Internet]. 2023.

762. Republic of Malta. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

763. Ministry of Health. Communicable Disease Control Strategy For Malta [Internet]. Malta; 2013 [cited 2022 Aug 10].

764. Government of Malta. Notifiable Disease. Infectious Disease Prevention & Control Unit [Internet]. 2021 [cited 2022 Aug 10].

765. Republic of Malta. Public Health Act. Chapter 465. Malta; 2007 Jun.

766. Infectious Disease Prevention and Control Unit Malta. Monthly Confirmed Notifiable Infectious Diseases – June 2018. Malta: Health Promotion and Disease Prevention Directorate; 2018 Jul.

767. Republic of the Marshall Islands. Ministry Of Health And Human Services [Internet]. 2023 [cited 2023 Jan 19].

768. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of the Marshall Islands, Mission report: 23-26 September 2019. No.9789240008168. Geneva: World Health Organization; 2020.

769. Islamic Republic of Mauritania. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

770. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la République islamique de Mauritanie Rapport de mission, mars 2017 [Joint External Evaluation of IHR Core Capacities of the Islamic Republic of Mauritania, Mission report, March 2017]. No. WHO/WHE/CPI/2017.28. Geneva: World Health Organization; 2017.

771. Ministry of Health Mauritania. Statistical Yearbook 2014. Monitoring and Evaluations. 2014.

772. Islamic Republic of Mauritania. Décret n° 361-2019/PM fixant les attributions du Ministre de la Santé et l'organisation de l'administration centrale de son département. [Decree No. 361-2019/PM Setting the powers of the Minister of Health and the organization of the central administration of his department]. Mauritania; 2019.

773. Republic of Mauritius. Ministry of Health and Wellness [Internet]. 2023 [cited 2023 Jan 19].

774. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Mauritius, Mission report 29 October-2 November 2018. No. WHO/WHE/CPI/2019.55. Geneva: World Health Organization; 2019.

775. Republic of Mauritius. Public Health Act; amended Act No 1 2020. Report No.47/1925. Mauritius; 1925 Dec.

776. Republic of Mauritius. New Cases Of Certain Notifiable Diseases Reported To Sanitary Authorities 1987-2007 [Internet]. Mauritius; 2007 [cited 2023 Jan 5].

777. Mexico. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

778. Ministry of Health Mexico. Boletín Epidemiológico Sistema Nacional de Vigilancia Epidemiológica Sistema Único de Información [Epidemiological Bulletin National Epidemiological Surveillance System Single Information System]. Report No. Week 1. Mexico; 2022 [cited 2023 Jan 19].

779. Government of Mexico. Norma Para La Vigilancia epidemiológica [Standard for Epidemiological Surveillance.]. Report No.NOM-017-SSA2-2012. Mexico; 2013 Feb.

780. Government of Mexico. Disaster Health Care Manual [Internet]. National Center for Disease Control and Preventive Programs. 2017 [cited 2023 Jan 19].

781. Secretary of Health Government of Mexico. National Plan for Preparedness and Response to the Intensification of Seasonal Influenza or to an Influenza Pandemic [Internet]. Mexico: National Center for Disease Control and Preventive Programs; 2018 Aug [cited 2023 Jan 19].

782. Government Of The Federated States Of Micronesia. Department of Health and Social Affairs [Internet]. 2023 [cited 2023 Jan 19].

783. Federated States of Micronesia. Government Of The Federated States Of Micronesia [Internet]. 2023 [cited 2023 Jan 19].

784. World Health Organization. Joint external evaluation of IHR core capacities of the Federated states of Micronesia, Mission report: 13-17 August 2018. No. WHO/WHE/CPI/2018.34. Geneva: World Health Organization; 2019.

785. Federated States of Micronesia. Title 41 Public Health, Safety And Welfare [Internet]. 2014 [cited 2023 Jan 19].

786. Government Of The Federated States Of Micronesia. FSM COVID-19 Response Framework [Internet]. MICRONESIA; 2020 Apr [cited 2022 Aug 10].

787. Republic of Moldova. Ministry of Health [Internet]. Ministerul Sănătății RM; 2023 [cited 2023 Jan 19].

788. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Moldova, Mission report: 1-6 October 2018. No. WHO/WHE/CPI.2019.54. Geneva: World Health Organization; 2019.

789. National Agency for Public Health. Epidemiological Bulletin Republic of Moldova [Internet]. 2022 [cited 2023 Jan 19].

790. Republic of Moldova. Regarding State Supervision Of Public Health law No. 10 [Internet]. 2009 [cited 2023 Jan 19].

791. Principality of Monaco. Print this page Ministry of Health and Social Affairs [Internet]. 2023 [cited 2023 Jan 19].

792. Ministry of Health and Social Affairs Monaco. An exemplary Public Health system [Internet]. 2023 [cited 2023 Jan 19].

793. Principality of Monaco. Ordinance no. 3.153 of 02/24/2011 rendering enforceable the International Health Regulations (2005) adopted by the fifty-eighth World Health Assembly on May 23, 2005. Monaco; 2011.

794. Mongolia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

795. World Health Organization. Joint external evaluation of IHR core capacities of Mongolia, Mission report: May 12-19, 2017. No. WHO/WHE/CPI/REP/2017.51. Geneva: World Health Organization; 2017.

796. Mongolia. National Centre for Communicable Disease [Internet]. 2023 [cited 2023 Jan 19].

797. Ministry of Health Mongolia. Health Statistical Information December 2022 [Internet]. 2023 [cited 2023 Jan 19].

798. Montenegro. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

799. Montenegro. Institute for Public Health [Internet]. 2023 [cited 2023 Jan 19].

800. World Health Organization. Joint external evaluation of IHR core capacities of Montenegro, Mission report: 27-31 May 2019. No.9789240018228. Geneva: World Health Organization; 2021.

801. Institute Of Public Health Of Montenegro. Health Statistical Yearbook 2018 of Montenegro. Montenegro; 2020.

802. Institute Of Public Health Of Montenegro. Mjesečni Izvještaj O Kretanju Zaraznih Bolesti [Monthly Report on the Trend of Infectious Diseases] [Internet]. 2021 [cited 2022 Aug 12].

803. Montenegro. The Law On The Prevention Of Population Against Infective Diseases. No.12/2018. Montenegro; 2018 Feb.

804. Kingdom of Morocco. Ministry of Health and Social Protection [Internet]. 2023 [cited 2023 Jan 19].

805. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Morocco, Mission report: 20-24 June 2016. No. WHO/WHE/CPI/2017.3. Geneva: World Health Organization; 2017.

806. Ministry of Health and Social Protection Morocco. Bulletin of epidemiology and public health No 79 [Internet]. 2022 [cited 2023 Jan 19].

807. Morocco. Institut Pasteur Morocco [Internet]. 2015 [cited 2023 Jan 19].

808. Kingdom of Morocco. Order of the Minister of Public Health No. 683-95. Morocco; 2010.

809. Republic of Mozambique. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

810. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Mozambique, Mission report: 18-22 April 2016. Report No. WHO/WHE/CPI/2017.19. Geneva: World Health Organization; 2017.

811. Ministry of Health Mozambique. Strategic Plan for the Health Sector 2014 - 2019. Mozambique; 2016 Nov.

812. Ministry of Health Mozambique. Anuário Estatístico de Saúde 2021 [Health Statistical Yearbook 2021]. Mozambique; 2022 Mar.

813. Republic of the Union of Myanmar. Ministry of Health [Internet]. 2022 [cited 2022 Aug 12].

814. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of the Union Myanmar, Mission report: 3-9 May 2017. Report No. WHO/WHE/CPI/REP/2018.5. Geneva: World Health Organization; 2018.

815. Republic of the Union of Myanmar. National Action Plan For Health Security 2018-2022. Myanmar: Ministry of Health and Sports; 2018 Oct.

816. Republic of the Union of Myanmar. The Prevention and Control of Communicable Diseases Law. No.1/95.Myanmar: The State Law and Order Restoration Council; 1995 Mar.

817. Republic of the Union of Myanmar. The Law Amending the Prevention and Control of Communicable Diseases Law. No.16 / 2011. Myanmar: The State Peace and Development Council; 2011 Jan.

818. Republic of the Union of Myanmar. Public Health Statistics (2014‑2016). Myanmar: Ministry of Health and Sports; 2017 Sep.

819. Republic of Namibia. Ministry of Health and Social Services [Internet]. 2023 [cited 2023 Jan 19].

820. World Health Organization. Joint external evaluation of IHR core capacities of Republic of Namibia, Mission report: 28 November to 22 December 2016. No. WHO/WHE/CPI/REP/2017.36. Geneva: World Health Organization; 2017.

821. Republic of Namibia. Public and Environmental Health Act 1 of 2015; amendment 17 September 2020. No.GN 230/2020. Namibia; 2015 Apr.

822. Republic of Nauru. The Government of the Republic of Nauru [Internet]. 2023 [cited 2023 Jan 19].

823. Republic of Nauru. Health Sector Strategic Plan 2010–2015. Nauru: Ministry of Health and Medical Service; 2010.

824. Republic of Nauru. Notification of Infectious and Contagious Diseases Ordinance 1923; amendment 26 Sept 2014. Nauru; 2011 Nov.

825. Federal Democratic Republic of Nepal. स्वास्थ्य तथा जनसङ्ख्या मन्त्रालय [Ministry of Health and Population] [Internet]. 2022 [cited 2022 Aug 12].

826. Ministry of Health and Population Nepal. Surveillance Of Communicable Disease Program [Internet]. 2022 [cited 2023 Jan 19].

827. Federal Democratic Republic of Nepal. Early Warning and Reporting System (EWARS) - Weekly Bulletin: Nepal 52nd Epidemiological Week, 2022 [Internet]. 2023 [cited 2023 Jan 19].

828. Federal Democratic Republic of Nepal. Infectious Disease Act 1964; amendment 16 Nov 2020. Nepal: Nepal Law Commission; 1964 Feb.

829. Nepal Health Research Council. Review of Existing Diseases Surveillance System in Nepal from Climate Change Perspective [Internet]. 2019 [cited 2023 Jan 19].

830. Government of the Netherlands. Ministry of Health, Welfare and Sport [Internet]. 2023 [cited 2023 Jan 19].

831. Ministry of Health, Welfare and Sport Netherlands. Welke infectieziekten zijn meldingsplichtig? [Which infectious diseases are notifiable?] [Internet]. Netherlands: National Institute for Public Health and the Environment; 2022 Dec [cited 2023 Jan 5].

832. Editors Infectious Diseases Bulletin Netherlands. Public Health Act notifications up to and including week 36, 2022 / IB 10-2022 [Internet]. 2022 [cited 2023 Jan 19].

833. Ministry of Health, wellbeing and sports Netherlands. National guidance for infectious disease crises [Internet]. National Institute for Health and Environment. 2018 Nov 11 [cited 2022 Aug 21].

834. Kingdom of the Netherlands. Wet publieke gezondheid [Public Health Act]. Netherlands; 2021 Feb.

835. New Zealand. Manatū Hauora Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

836. World Health Organization. Joint external evaluation of IHR core capacities of New Zealand, Mission report: 26-30 November 2018. No. WHO/WHE/CPI/2019.63. Geneva: World Health Organization; 2019.

837. Ministry of Health New Zealand. List of diseases notifiable by health practitioners and laboratories to the Medical Officer of Health [Internet]. New Zealand; 2022 [cited 2022 Aug 13].

838. New Zealand. Health Act 1956; Version as at 1 July 2022. No.1956 No 65. New Zealand: Ministry of Health; 1956 Oct.

839. Ministry of Health New Zealand. Monthly Notifiable Disease Surveillance Report - Nov 2022 [Internet]. Public Health Surveillance. 2023 [cited 2023 Jan 19].

840. Institute of Environmental Science and Research. Guidelines for the Investigation and Control of Disease Outbreaks [Internet]. Porirua, New Zealand; 2012 Apr [cited 2022 Aug 13].

841. Republic of Nicaragua. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

842. Ministry of Health Nicaragua. Epidemiological Bulletin of the Week No. 2 [Internet]. Nicaragua; 2022 Feb [cited 2022 Aug 13].

843. Republic of Nicaragua. General Health Law. No. 423. Nicaragua; 2002 May.

844. Republic of the Niger. Ministry of Public Health, Population and Social Affairs [Internet]. 2022 [cited 2022 Aug 13].

845. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la République du Niger Rapport de mission: 16-20 juillet 2018 [Joint external evaluation of the RSI core capacities of the Republic of Niger, Mission report: July 16-20, 2018]. No. WHO/WHE/CPI/2018.33. Geneva: World Health Organization; 2018.

846. Devex. Ministry Of Public Health (Niger) [Internet]. 2023 [cited 2023 Jan 19].

847. Federal Republic of Nigeria. Federal Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

848. World Health Organization. Joint external evaluation of IHR core capacities of the Federal Republic of Nigeria, Mission report: June 11-20, 2017. No. WHO/WHE/CPI/REP/2017.46. Geneva: World Health Organization; 2017.

849. Federal Republic of Nigeria. Nigeria Centre for Disease Control [Internet]. 2022 [cited 2022 Aug 13].

850. Nigeria Centre for Disease Control. Weekly Epidemiological Report August Week 32 [Internet]. 2022 [cited 2023 Jan 19].

851. Isere EE, Fatiregun AA, Ajayi IO. An overview of disease surveillance and notification system in Nigeria and the roles of clinicians in disease outbreak prevention and control. Niger Med J. 2015;56(3):161.

852. Federal Republic of Nigeria. National Health Act, 2014. Act No. 8 of 2014. Nigeria; 2014 Oct.

853. Federal Ministry of Health - Nigeria Centre for Disease Control. National Technical Guidelines For Integrated Disease Surveillance And Response; updated 2022. 3rd Edition. Nigeria; 2019.

854. Government of Niue. Ministries. Office of the Secretary to Government [Internet]. 2022 [cited 2022 Aug 13].

855. Niue. Public Health Act 1965. No: 1965/24. Niue: Government of Niue; 1965 Jan.

856. Government of Niue. Health Strategic Plan 2011 - 2021. Niue; 2011.

857. Park JJ, Lim AY, Ahn HS, Kim AI, Choi S, Oh DHW, et al. Systematic review of evidence on public health in the Democratic People's Republic of Korea. BMJ Global Health. 2019;4(2):e001133.

858. Democratic People's Republic of Korea. 인민보건법 2012 [People's Public Health Law of the Democratic People's Republic of Korea (2012)]. Korea: Law and North Korea;

2012 Apr.

859. Republic of North Macedonia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

860. World Health Organization. Joint external evaluation of IHR core capacities Republic of North Macedonia, Mission report: 11-15 March 2019. No. WHO/WHE/CPI/2019.59. Geneva: World Health Organization; 2017.

861. EWARN North Macedonia. Weekly report on infectious diseases in Macedonia Week 50-2019 [Internet]. 2019 [cited 2023 Jan 19].

862. Republic of North Macedonia. Law On The Protection Of The Population Against Infectious Diseases no. 37/16. 2016 [Internet]. North Macedonia. 2016 [cited 2023 Jan 19].

863. Ministry of Health and Care Services Norway. Ministry of Health and Care Services [Internet]. 2022 [cited 2022 Aug 14].

864. Ministry of Health and Care Services Norway. National Health Preparedness Plan [Internet]. Version 2.0. Norway: Norwegian Government Security and Service Organisation; 2014 Jun.

865. Kingdom of Norway. Lov om vern mot smittsomme sykdommer Smittevernloven [Act on Protection against Infectious Diseases] [Internet]. Norway: Ministry of Health and Welfare; 2023 Jan [cited 2023 Jan 5].

866. Norwegian Institute of Public Health. Notifiable diseases in the Norwegian Surveillance System for Communicable Diseases [Internet]. Norway; 2019 Jul [cited 2023 Jan 5].

867. Norwegian Institute of Public Health. Meldingssystem for smittsomme sykdommer [Notification system for infectious diseases] [Internet]. 2023 [cited 2023 Jan 19].

868. Norwegian Institute of Public Health. Når skal det varsles om smittsomme sykdommer? [When must notifications be made about infectious diseases?] [Internet]. 2022 [cited 2023 Jan 19].

869. Sultanate of Oman. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

870. World Health Organization. Joint external evaluation of IHR core capacities of the Sultanate of Oman, Mission report: 2-7 April, 2017. No. WHO/WHE/CPI/REP/2017.59. Geneva: World Health Organization; 2017.

871. Sultanate of Oman Ministry of Health. Annual Health Report 2020 [Internet]. Oman: Department of Information & Statistics; 2021 [cited 2023 Jan 19].

872. Sultanate of Oman. The Law on the Control of Communicable Diseases Promulgated by Royal Decree. No.73/92. Oman: Ministry of Legal Affairs; 2020.

873. Islamic Republic of Pakistan. Ministry of National Health Services Regulations & Coordination [Internet]. 2023 [cited 2023 Jan 19].

874. Islamic Republic of Pakistan. National Institutes of Health [Internet]. 2023 [cited 2023

Jan 19].

875. World Health Organization. Joint external evaluation of IHR core capacities of the Islamic Republic of Pakistan, Mission report: 27 April - 6 May 2016. No. WHO/WHE/CPI/2017.9. Geneva: World Health Organization; 2017.

876. Ministry of National Health Services Regulations & Coordination. Notification of Priority Diseases in Pakistan [Internet]. Pakistan: Government of Pakistan; 2017 Dec [cited 2023 Jan 5].

877. Islamic Republic of Pakistan. The West Pakistan Epidemic Diseases Act. No.36-1958. Pakistan; 1958 May.

878. Pakistan Ministry of National Health Services Regulations & Coordination. National Level Integrated Dashboard [Internet]. Pakistan; 2022 [cited 2023 Jan 19].

879. Republic of Palau. Ministry of Health and Human Services [Internet]. 2023 [cited 2023 Jan 19].

880. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Palau, Mission report 29 July-2 August 2019. No.9789240008144. Geneva: World Health Organization; 2020.

881. Ministry of Health Republic of Palau. National Health Profile 2013 [Internet]. Republic of Palau; 2014 [cited 2022 Aug 14].

882. Republic of Palau. Ministry of Health Annual Report [Internet]. Palau; 2014 [cited 2022 Aug 14].

883. Republic of Panama. Ministry of Health [Internet]. 2023 [cited 2023 Jan 19].

884. Republic of Panama Ministry of Health. Weekly Epidemiological Bulletin 2022 - Week No 47 [Internet]. 2022 [cited 2023 Jan 19].

885. Republic of Panama. Executive Decree No 268 That Determines Mandatory Notification Health Problems, Points Out The Procedures for Notification and Establish Sanctions. Panama; 2001.

886. Papua New Guinea. Ministry of Health. National Department Of Health [Internet]. 2023 [cited 2023 Jan 19].

887. Independent State of Papua New Guinea. Public Health Act 1973. No.226. Papua New Guinea; 1973.

888. Government of Papua New Guinea. National Health Plan 2021-2030. No.978-9980-86-241-9. Papua New Guinea: National Department of Health; 2021 Jun.

889. Republic of Paraguay. Ministry of Health and Social Welfare [Internet]. 2022 [cited 2022 Aug 14].

890. Ministry of Health and Social Welfare Paraguay. Weekly Epidemiological Bulletin - 49 [Internet]. Paraguay; 2022 [cited 2023 Jan 20].

891. Ministry of Health and Social Welfare Paraguay. Guía Nacional de Vigilancia y Control de Enfermedades [National Surveillance Guide and Disease Control]. No.978-99925-11-03-9. Paraguay: Dirección General de Vigilancia de la Salud; 2022 May.

892. Republic of Paraguay. Law No. 836 Sanitary Code. Paraguay; 1980.

893. Republic of Peru. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

894. Republic of Peru. National Institute of Health [Internet]. 2023 [cited 2023 Jan 20].

895. Ministry of Health Peru. Notificación de brotes, epidemias y otros eventos de importancia para la salud pública [Notification of outbreaks, epidemics and other events of importance to public health]. Lima, Perú: Dirección General de Epidemiología; 2012.

896. Ministry of Health Peru. Herramientas para la vigilancia epidemiológica [Tools for epidemiological surveillance] [Internet]. National Center for Disease Control, Prevention, and Epidemiology. Peru; 2023 [cited 2023 Jan 5].

897. Ministry of Health Peru. Notificacion De Enfermedades Y Eventos Sujetos A Vigilancia Epidemiologica En Salud Publica [Notification Of Illnesses And Events Subject To Surveillance Epidemiology In Public Health]. Peru; 2013.

898. Republic of Peru. Ley Nº 26842 – Ley General De Salud [Law No. 26842 – General Health Law]. Peru; 1997.

899. Republic of Peru. Epidemiological Bulletin 51 - 2022. National Center for Disease Control, Prevention, and Epidemiology [Internet]. 2023 [cited 2023 Jan 20].

900. Republic of the Philippines. Department of Health [Internet]. 2023 [cited 2023 Jan 20].

901. World Health Organization. Joint external evaluation of IHR core capacities Republic of the Philippines, Mission report: 10-14 September 2018. No. WHO/WHE/CPI/2019.57. Geneva: World Health Organization; 2019.

902. Republic of the Philippines. The 2020 Revised Implementing Rules and Regulations of Republic Act No. 11332, or the Mandatory Reporting of Notifiable Diseases and Health Events of Public Health Concern Act. Philippines; 2018.

903. Republic of the Philippines Department of Health. 2022 EDCS Weekly Surveillance Report No. 51. Epidemic-prone Disease Case Surveillance [Internet]. 2023 [cited 2023 Jan 20].

904. Republic of Poland. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

905. Ministry of Health Poland. Zakład Epidemiologii Chorób Zakaźnych I Nadzoru Pracownia Monitorowania I Analizy Sytuacji Epidemiologicznej [Department Of Epidemiology Of Infectible Diseases And Supervision Laboratory For Monitoring And Analysis Of Epidemiological Situation] [Internet]. 2022 [cited 2022 Aug 14].

906. Ministry of Health Poland. Monitoring and Analysis of the Epidemiological Situation

[Internet]. Poland; 2022 [cited 2022 Aug 14].

907. Republic of Poland. On preventing and combating infections and infectious diseases in humans No. 234 item 1570. Poland; 2008.

908. Republic of Portugal. Directorate-General for Health [Internet]. 2023 [cited 2023 Jan 20].

909. Directorate-General for Health Portugal. Public Health Emergency Center [Internet]. National Health Service. 2023 [cited 2023 Jan 20].

910. Ministry of Health Republic of Portugal. Lista de doenças transmissíveis de notificação obrigatória [List of notifiable communicable diseases]. No.Despacho nº 15385-A. Portugal; 2016 Dec.

911. Ministry of Health Portugal. Doenças De Declaração Obrigatória 2013-2016 [Diseases Of Mandatory Declaration 2013-2016]. Vol 1. Lisbon, Portugal; 2017 Dec.

912. Republic of Portugal. Law nº 81/2009 of 21 August. Assembly of the Republic; 2009.

913. Republic of Portugal. Order No. 15385-A/2016 of December 21st. Director General of Health. 2016.

914. State of Qatar. Ministry of Public Health [Internet]. 2023 [cited 2023 Jan 20].

915. World Health Organization. Joint external evaluation of IHR core capacities of the State of Qatar, Mission report: 29 May - 2 June 2016. No. WHO/WHE/CPI/2017.6. Geneva: World Health Organization; 2017.

916. State of Qatar. Decree Law No. 17 of 1990 on Protection from Infectious Diseases. Qatar; 1990.

917. Government of Qatar. Communicable Disease Reports [Internet]. Qatar Open Data Portal. 2018 [cited 2023 Jan 20].

918. Romania. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

919. Romania. National Institute Of Public Health [Internet]. 2023 [cited 2023 Jan 20].

920. Romania. National Center for Surveillance and Control of Communicable Diseases [Internet]. 2023 [cited 2023 Jan 20].

921. National Center for Surveillance and Control of Communicable Diseases Romania. Analiza bolilor transmisibile aflate în supraveghere Raport pentru anul 2018 [Analysis of communicable diseases under surveillance Report for the year 2018]. Romania; 2019.

922. Romania. Privind stabilirea metodologiei de raportare şi de colectare a datelor pentru supravegherea bolilor transmisibile [Regarding the establishment of the reporting and data collection methodology for the surveillance of communicable diseases] [Internet]. Romania: National Center for Surveillance and Control of Communicable Diseases; 2007 Jun [cited 2023 Jan 5]. Report No.HG 589 2007.

923. Russian Federation. Ministry of Health [Internet]. 2022 [cited 2022 Aug 15].

924. Federal Service for Supervision of Consumer Rights Protection and Human Welfare. Инфекционная заболеваемость в Российской Федерации за январь-декабрь 2018г. [Infectious incidence in the Russian Federation for January-December 2018]. Russia; 2019.

925. Russian Federation. ОБ УТВЕРЖДЕНИИ ФОРМ ФЕДЕРАЛЬНОГО СТАТИСТИЧЕСКОГО НАБЛЮДЕНИЯ С УКАЗАНИЯМИ ПО ИХ ЗАПОЛНЕНИЮ ДЛЯ ОРГАНИЗАЦИИ ФЕДЕРАЛЬНОЙ СЛУЖБОЙ ПО НАДЗОРУ В СФЕРЕ ЗАЩИТЫ ПРАВ ПОТРЕБИТЕЛЕЙ И БЛАГОПОЛУЧИЯ ЧЕЛОВЕКА ФЕДЕРАЛЬНОГО СТАТИСТИЧЕСКОГО НАБЛЮДЕНИЯ ЗА САНИТАРНЫМ СОСТОЯНИЕМ СУБЪЕКТА РОССИЙСКОЙ ФЕДЕРАЦИИ [On Approval Of Forms Of Federal Statistical Observation With Instructions For Completing Them For The Federal Service For Supervision Of Consumer Rights Protection And Human Welfare To Organize Federal Statistical Observation Of The Sanitary Condition Of A Subject Of The Russian Federation]. Russia: Ministry of Justice;2020.

926. Russian Federation. об Оптимизации Противоэпидемической Работы И Утверждении Формы Акта Эпидемиологического Расследования Очага Инфекционной (паразитарной) Болезни С Установлением Причинно-следственной Связи" [On The Optimization Of Anti-epidemic Work And The Approval Of The Form Of The Act Of The Epidemiological Investigation Of The Focus Of An Infectious (Parasitic) Disease With The Establishment Of A Causal Relationship]. Russia: Ministry of Justice; 2010 Nov.

927. Republic of Rwanda. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

928. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Rwanda, Mission report: 14-18 May 2018. No. WHO/WHE/CPI/REP/2018.22. Geneva: World Health Organization; 2018.

929. Republic of Rwanda. Fourth Health Sector Strategic Plan July 2018 – June 2024 [Internet]. 2018 [cited 2023 Jan 20].

930. Ministry of Health Republic of Rwanda. Annual Health Statistics Booklet 2016 [Internet]. 2017 [cited 2023 Jan 20].

931. Republic of Rwanda. Laws and Regulations [Internet]. 2023 [cited 2023 Jan 20].

932. Independent State of Samoa. Ministry of Health [Internet]. 2022 [cited 2022 Aug 15].

933. Indo-Pacific Centre for Health Security. Pacific Health Security Scoping Mission Samoa Country Report. Samoa; 2018.

934. Independent State of Samoa. Ministry of Health Act No 19. Samoa; 2006.

935. White P, Saketa S, Durand A, Vaai-Nielsen S, Leong-Lui TA, Naseri T, et al. Enhanced surveillance for the third United Nations conference on Small Island developing states, Apia, Samoa, September 2014. Western Pacific surveillance and response journal: WPSAR. 2017;8(1):15.

936. Ministry of Health, Samoa. Bulletin Vol.2 Issue HIV, STI, and TB Programme [Internet]. Samoa; February 2019 [cited 2023 Jan 20].

937. Ministry of Health Samoa. National Epidemic & Pandemic Influenza Preparedness and Response Plan. Samoa; 2022.

938. Republic of San Marino. Secretariat of State for Health and Social Security [Internet]. 2022 [cited 2022 Aug 16].

939. Republic of San Marino. Social Security Institute [Internet]. 2022 [cited 2022 Aug 16].

940. Republic of San Marino. Ordinanze del Segretario di Stato per la Sanità e la Sicurezza Sociale [Ordinances of the Secretary of State for Health and Social Security] [Internet]. San Marino; 2022 [cited 2022 Aug 16].

941. Republic of San Marino. Notifiche Di Malattie Infettive E Diffuse [Notification Of Infectious And Diffuse Diseases] [Internet]. San Marino; 2021 [cited 2022 Aug 16].

942. Republic of San Marino. Bulletin IV quarter 2022 [Internet]. 2023 [cited 2023 Jan 20].

943. Democratic Republic of São Tomé and Príncipe. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

944. World Health Organization. Joint external evaluation of IHR core capacities of the Democratic Republic of São Tomé and Príncipe, Mission report: 13-17 May 2019. No. WHO/WHE/CPI/2019.10. Geneva: World Health Organization; 2019.

945. Ministry of Health São Tomé and Príncipe. Anuário Estatístico da Saúde 2020 [Health Statistical Yearbook 2020]. São Tomé and Príncipe; 2021 Feb.

946. São Tomé and Príncipe. Lei n.º 09/2018 Lei Base de Saúde [Basic Health Law]. São Tomé and Príncipe; 2018.

947. Ministry of Health São Tomé and Príncipe. Manual De Procedimentos Para O Serviço Nacional De Saúde [Procedures Manual For The National Health Service]. São Tomé and Príncipe; 2019.

948. Kingdom of Saudi Arabia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

949. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Saudi Arabia, Mission report: 12-16 March 2017. No. WHO/WHE/CPI/2017.25. Geneva: World Health Organization; 2017.

950. Kingdom of Saudi Arabia Ministry of Health. Reported Cases of Notifiable Communicable Diseases by Region 2019 [Internet]. Saudi Arabia; 2019 [cited 2022 Aug 16].

951. Kingdom of Saudi Arabia. Health System Royal Decree No. M/11. Saudi Arabia; 2002.

952. Kingdom of Saudi Arabia Ministry of Health. Public Health Surveillance Technical

Guidelines. Saudi Arabia; 2017.

953. Republic of Senegal. Ministry of Health and Social Action [Internet]. 2023 [cited 2023 Jan 20].

954. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Senegal, Mission report: 18 November-2 December 2016. No. WHO/WHE/CPI/2017.31. Geneva: World Health Organization; 2018.

955. Ministry of Health and Social Action Senegal. Surveillance Integree De La Maladie Et La Riposte [Integrated Disease Surveillance And Response]. Senegal; 2020.

956. Republic of Senegal. Decree nº2020-936 on the organization of the Ministry of Health and Social Action. 2020 Apr 3.

957. Republic of Serbia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

958. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Serbia, Mission report: 8-12 October 2018. No. WHO/WHE/CPI.2019.36. Geneva: World Health Organization; 2019.

959. Ministry of Health. Godišnji izveštaj o zaraznim bolestima u Republici Srbiji za 2020. godinu [Annual report on infectious diseases in the Republic of Serbia for 2020]. Serbia: Institute for Public Health of Serbia; 2022 Aug.

960. Republic of Serbia. Закон о заштити становништва од заразних болести [Law on the Protection of the Population from Infectious Diseases]. No.15/2016-31, 68/2020-4, 136/2020-3. Serbia; 2020 Aug.

961. Republic of Serbia. Правилник о пријављивању заразних болести и посебних здравствених питања [Rulebook on reporting infectious diseases and special health issues]. No. 44/2017-11, 58/2018-66. Serbia; 2018.

962. Republic of Serbia. Form 1a - Report of suspected misuse of a biological agent. 2017.

963. Republic of Seychelles. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

964. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Seychelles, Mission report: 5-9 March 2018. No. WHO/WHE/CPI/REP/2018.21. Geneva: World Health Organization; 2018.

965. Republic of Seychelles. Public Health Act (Act 25 of 1959). Seychelles; 2012.

966. Ministry of Health Seychelles. The National Health Strategic Plan 2022-2026. Seychelles; 2022.

967. Ministry of Health, Seychelles. Vital Statistics Report 2021 [Internet]. 2022 [cited 2022 Aug 16].

968. Republic of Sierra Leone. Ministry of Health and Sanitation [Internet]. 2022 [cited 2022 Aug 16].

969. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Sierra Leone, Mission report: 31 October - 4 November 2016. No. WHO/WHE/CPI/2017.16. Geneva: World Health Organization; 2017.

970. Republic of Sierra Leone. Public Health Ordinance Act No 23. Sierra Leone; 1960.

971. Republic of Sierra Leone. The Public Health (Amendment) Act No 10. Sierra Leone; 2014.

972. Ministry of Health and Sanitation Republic of Sierra Leone. National Health Sector Strategic Plan 2017 – 2021. Sierra Leone; 2017.

973. Ministry Of Health And Sanitation Republic of Sierra Leone. Weekly National Epidemiological Bulletin Week 52, 21st to 27th Dec. 2020 [Internet]. 2021 [cited 2022 Aug 16].

974. Ministry Of Health And Sanitation Republic of Sierra Leone. National Community Health Worker Policy 2016-2020 [Internet]. Sierra Leone; 2016 [cited 2022 Aug 16].

975. Republic of Singapore. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

976. World Health Organization. Joint external evaluation of IHR core capacities of Singapore, Mission report: 16-20 April 2018. No. WHO/WHE/CPI/REP/2018.25. Geneva: World Health Organization; 2018.

977. Communicable Diseases Division. Weekly Infectious Disease Bulletin Week 52 25 - 31 Dec 2022 [Internet]. Ministry of Health Singapore. 2023 [cited 2023 Jan 20].

978. Ministry of Health Singapore. List Of Infectious Diseases Legally Notifiable Under The Infectious Diseases Act. Singapore; 2022.

979. Republic of Singapore. Infectious Diseases Act 1976; Revised 2020. Singapore; 2021.

980. Ministry of Health Singapore. Pandemic Readiness And Response Plan For Influenza And Other Acute Respiratory Diseases [Internet]. Singapore; 2014 [cited 2022 Aug 16].

981. Ministry of Health. Communicable Diseases Surveillance Singapore 2017 [Internet]. Singapore; 2018 [cited 2022 Aug 16].

982. Slovakia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

983. Slovak Republic. Analysis of the epidemiological situation in the Slovak Republic, including the disease COVID-19 for November 2022 [Internet]. 2022 [cited 2023 Jan 20].

984. Slovakia. Zákon o ochrane, podpore a rozvoji verejného zdravia a o zmene a doplnení niektorých zákonov [Act on the Protection, Support and Development of Public Health and on Amendments and Supplements to Certain Acts]; last amendment 30 Dec 2022. Slovakia; 2007.

985. Slovak Republic. Pandemický Plán Pre Prípad Pandémie V Slovenskej Republike

[Pandemic Plan For A Pandemic In Slovak Republic] [Internet]. Slovakia; 2020 [cited 2022 Aug 16].

986. Republic of Slovenia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

987. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Slovenia, Mission report: 5-9 June, 2017. No. WHO/WHE/CPI/REP/2017.32. Geneva: World Health Organization; 2017.

988. National Institute of Public Health Slovenia. Definicije prijavljivih nalezljivih bolezni za namene epidemiološkega spremljanja [Definitions of notifiable communicable diseases for the purposes of epidemiological surveillance] [Internet]. Slovenia; 2020 [cited 2022 Aug 16].

989. National Institute of Public Health Slovenia. Number of reported diagnoses of infectious diseases by age, Slovenia, annually [Internet]. Data Portal. 2022 [cited 2023 Jan 20].

990. Ministry of Health. Epidemiological monitoring of infectious diseases in Slovenia in 2019 and 2020 [Internet]. 2022 [cited 2023 Jan 20].

991. National Institute of Public Health of the Republic of Slovenia. Health Statistical Yearbook 2020. Slovenia; 2021.

992. Republic of Slovenia. Zakon o nalezljivih boleznih [Infectious Diseases Act]; last amendment September 27, 2022. Slovenia; 1995.

993. Solomon Islands. Ministry of Health and Medical Services [Internet]. 2023 [cited 2023 Jan 20].

994. Craig AT, Joshua CA, Sio AR, Donoghoe M, Betz-Stablein B, Bainivalu N, et al. Epidemic surveillance in a low resource setting: lessons from an evaluation of the Solomon Islands syndromic surveillance system, 2017. BMC Public Health. 2018;18(1):1–10.

995. Ministry of Health and Medical Services. Solomon Islands National Health Strategic Plan 2016 - 2020. Solomon Islands; 2016.

996. Ministry of Health and Medical Services. Solomon Islands Country Report - Scoping Mission. Solomon Islands: Indo Pacific Centre for Health Security; 2018.

997. Ministry of Health and Medical Services. Statistical Health Core Indicator Report Solomon Islands 2018 [Internet]. 2019 [cited 2023 Jan 20].

998. Federal Republic of Somalia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

999. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Somalia, Mission report: 17-21 October 2016. No. WHO/WHE/CPI/2017.17. Geneva: World Health Organization; 2017.

1000. Lubogo M, Karanja MJ, Mdodo R, Elnossery S, Osman AA, Abdi A, et al. Evaluation of the electronic Early Warning and Response Network (EWARN) system in Somalia, 2017–2020. Confl Health. 2022;16(1):1–12.

1001. Federal Republic Of Somalia Ministry of Health and Human Services. Health Sector Strategic Plan 2017 - 2021. Somalia; 2021.

1002. Republic of South Africa. National Department of Health [Internet]. 2023 [cited 2023 Jan 20].

1003. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of South Africa, Mission report 27 November - 1 December 2017. No. WHO/WHE/CPI/REP/2018.10. Geneva: World Health Organization; 2018.

1004. National Institute for Communicable Diseases South Africa. Communicable diseases communiqué [Internet]. Vol. 21(9).Division of Public Health Surveillance and Response; 2022 Sep [cited 2023 Jan 5].

1005. Republic of South Africa. National Health Act: Regulations: Surveillance and control of notifiable medical conditions Act No 61 of 2003. South Africa; 2017.

1006. National Institute for Communicable Diseases. Notifiable Medical Conditions Case Definitions Flipchart [Internet]. Republic of South Africa. 2021 [cited 2023 Jan 20].

1007. Republic of Korea. Ministry of Health and Welfare [Internet]. 2023 [cited 2023 Jan 20].

1008. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Korea, Mission report: 28 August - 1 September 2017. No. WHO/WHE/CPI/2017.65. Geneva: World Health Organization; 2018.

1009. Korea Centers for Disease Control and Prevention. 2022 Infectious Disease Surveillance Week 52 [Internet]. 2022 [cited 2023 Jan 20].

1010. Korea Centers for Disease Control and Prevention. 법정감염병(등급별) [Notifiable Infectious Diseases (by Grade)] [Internet]. South Korea; 2023 [cited 2023 Jan 20].

1011. Ministry of Health and Welfare. Infectious Disease Control And Prevention Act No. 13639. South Korea; 2015.

1012. Ministry of Health and Welfare South Korea. 제2차 감염병 예방관리 기본계획(2018~2022) [Second Infectious Disease Prevention and Management Basic Plan (2018~2022)] [Internet]. South Korea; 2018 [cited 2023 Jan 5].

1013. Republic of South Sudan. Ministry of Health [Internet]. 2022 [cited 2022 Aug 18].

1014. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of South Sudan, Mission report: 15-20 October 2017. No. WHO/WHE/CPI/REP/2018.4. Geneva: World Health Organization; 2018.

1015. Ministry of Health Republic of South Sudan. National Health Policy 2016-2026 [Internet]. South Sudan; 2016 [cited 2022 Aug 18].

1016. Kingdom of Spain. Ministry of Health [Internet]. 2023 [cited 2023 Jan 20].

1017. Ministry of Health Spain. Sistema Nacional de Alerta Precoz y Respuesta Rápida (SIAPR) [National Early Warning and Rapid Response System (SIAPR)] [Internet].

Spain; 2022 [cited 2022 Aug 18].

1018. Kingdom of Spain. Orden Por la que se modifican los anexos I, II y III del Real Decreto 2210/1995, de 28 de diciembre, por el que se crea la Red Nacional de Vigilancia Epidemiológica, relativos a la lista de enfermedades de declaración obligatoria, modalidades de declaración y enfermedades endémicas de ámbito regional. [Order Which modifies annexes I, II and III of Royal Decree 2210/1995, of December 28, which creates the National Epidemiological Surveillance Network, relating to the list of notifiable diseases, declaration modalities and regional endemic diseases.]. No.SSI/445/2015. Spain: Ministry of Health, Social Services and Equality; 2015 Mar.

1019. National Epidemiology Center, Carlos III Health Institute. Boletín Semanal en Red. Número 52. Año 2022. Centro Nacional de Epidemiología [Weekly Online Bulletin. Number 52. Year 2022. National Center for Epidemiology] [Internet]. 2022 [cited 2023 Jan 20].

1020. Carlos III Health Institute Spain. Departamento De Enfermedades Transmisibles [Department Of Communicable Diseases] [Internet]. 2022 [cited 2022 Aug 18].

1021. Democratic Socialist Republic of Sri Lanka. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1022. World Health Organization. Joint external evaluation of IHR core capacities of the Democratic Socialist Republic of Sri Lanka, Mission report: June 19-23, 2017. No. WHO/WHE/CPI/REP/2017.33. Geneva: World Health Organization; 2017.

1023. Epidemiology Unit, Ministry of Health, Nutrition & Indigenous Medicine Sri Lanka. Weekly Epidemiological Report Vol 49 No 51 [Internet]. 2022 [cited 2023 Jan 21].

1024. Ministry of Justice - Sri Lanka. Quarantine And Prevention Of Diseases; Amendment Act 12 of 2005. Sri Lanka; 1897.

1025. Epidemiology Unit, Ministry of Health Sri Lanka. List of Notifiable Diseases [Internet]. 2005 [cited 2023 Jan 21].

1026. Government of St. Kitts and Nevis. Saint Kitts and Nevis [Internet]. 2022 [cited 2023 Jan 21].

1027. Saint Kitts and Nevis. Public Health Act 22 of 1969; revised 31 December 2002. Saint Kitts and Nevis; 1970.

1028. Saint Kitts and Nevis. Public Health (Quarantine) Regulations No. 2 of 2020. Saint Kitts and Nevis; 2020.

1029. Saint Lucia. Ministry of Health, Wellness and Elderly Affairs [Internet]. 2023 [cited 2023 Jan 21].

1030. Saint Lucia. Public Health Act No. 8 of 1975. St Lucia; 1975.

1031. St Lucia. Public Health (Communicable and Notifiable Diseases) (Amendment) Regulations No 19. 2020.

1032. Saint Lucia. National Communicable Disease Surveillance Manual [Internet]. St Lucia;

2006 [cited 2023 Jan 21].

1033. St Vincent & The Grenadines. Ministry of Health, Wellness and the Environment [Internet]. 2023 [cited 2023 Jan 21].

1034. Rodriguez, Michael, Taylor Williamson, Abigail Vogus, Elizabeth Macgregor-Skinner, Donna-Lisa Pena, Wilson, Anneke, Sharon Nakhimovsky. Saint Vincent and the Grenadines Health Systems and Private Sector Assessment. Health Systems 20/20 project. Bethesda, MD: Abt Associates Inc.; 2012 Feb.

1035. Saint Vincent and the Grenadines. Public Health (Public Bodies Special Measures) Rules 2021 [Internet]. [cited 2023 Jan 21].

1036. Republic of the Sudan. Federal Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1037. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of the Sudan, Mission report: 9-13 October 2016. No. WHO/WHE/CPI/2017.15. Geneva: World Health Organization; 2017.

1038. Republic of the Sudan. 2008 قانون الصحة العامة القومى لسنة [National Public Health Act of 2008.]. Sudan; 2018.

1039. Sudan Health Observatory. قائمة التبليغ عن الأمراض [Disease Notification List] [Internet]. 2013 [cited 2023 Jan 21].

1040. Republic of Suriname. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1041. Republic of Suriname. Nationaal Plan Pandemische Paraatheid [National Pandemic Preparedness Plan]. Suriname; 2009.

1042. Republic of Suriname. Law Of Public Health 2012, no 109. Suriname; 2012.

1043. Kingdom of Sweden. Ministry of Health and Social Affairs [Internet]. 2023 [cited 2023 Jan 21].

1044. Kingdom of Sweden. The Public Health Agency of Sweden [Internet]. 2023 [cited 2023 Jan 21].

1045. The Public Health Agency of Sweden. Notifiable diseases [Internet]. Sweden; 2022 [cited 2022 Aug 19].

1046. Public Health Authority Sweden. Anmälningspliktiga sjukdomar – Årsrapporter 2021 [Notifiable diseases – Annual reports 2021]. Sweden; 2022.

1047. Kingdom of Sweden. Smittskyddslag (2004:168) [Infection Prevention Act (2004:168)]; amended 24 Dec 2022. Sweden; 2004.

1048. Syrian Arab Republic. Ministry of Health [Internet]. 2022 [cited 2022 Aug 19].

1049. Ministry of Health Syrian Arab Republic. Communicable Diseases Quarterly Report Forth Quarter (October to December 2013). EWARS Review. Syria; 2014.

1050. Ministry of Health Syria. Syrian Arab Republic: EWARS Weekly Epidemiological Bulletin Week No 26 [Internet]. World Health Organisation. Syria; 2022 [cited 2023 Jan 21].

1051. Republic of Tajikistan. Ministry of Health and Social Protection of Population [Internet]. 2022 [cited 2022 Aug 19].

1052. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Tajikistan Mission report: 21–25 October 2019. No.9789240018242. Geneva: World Health Organization; 2021.

1053. Republic of Tajikistan. Кодекс Здравоохранения Республики Таджикистан [Health Code of the Republic of Tajikistan] [Internet]. 2017 [cited 2023 Jan 21].

1054. Republic of Tajikistan. Мониторинг и Раннее Оповещение в Таджикистане октябрь 2017 [Monitoring and Early Warning in Tajikistan October 2017] [Internet]. Tajikistan; 2017 [cited 2023 Jan 21].

1055. United Republic of Tanzania. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1056. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Tanzania, Mission report: February 2016. No. WHO/HSE/GCR/2016.25. Geneva: World Health Organization; 2017.

1057. World Health Organization. Joint external evaluation of IHR core capacities of the United Republic of Tanzania - Zanzibar, Mission report: 22-28 April 2017. No. WHO/WHE/CPI/REP/2017.39. Geneva: World Health Organization; 2017.

1058. United Republic of Tanzania. The Public Health Act No 1. Tanzania; 2009 [cited 2023 Jan 21].

1059. Kingdom of Thailand. Ministry of Public Health [Internet]. 2022 [cited 2022 Aug 19].

1060. World Health Organization. Joint external evaluation of IHR core capacities of the Kingdom of Thailand, Mission report: June 26-30, 2017. No. WHO/WHE/CPI/REP/2017.38. Geneva: World Health Organization; 2017.

1061. Bureau of Epidemiology, Department of Disease Control Thailand [Internet]. National Disease Surveillance (Report 506). Ministry of Public Health. 2023 [cited 2023 Jan 27].

1062. Kingdom of Thailand. Communicable Disease Act. Thailand; 2003.

1063. Ministry of Public Health Thailand. Reporting Guidelines dangerous communicable disease and communicable diseases that need to be monitored According to the Communicable Disease Act B.E. 2558 (2015). Thailand; 2018.

1064. Ministry of Public Health Thailand. National Strategic Plan for Emerging Infectious Diseases 2017-2021. Thailand; 2016.

1065. Ministry of Public Health Thailand. คู่มือเครือข่ายห้องปฏิบัติการ โรคติดเชื้ออุบัติใหม่ ฉบับ

2558 [Laboratory Network Guide emerging infectious diseases 2015 edition]. Thailand; 2015.

1066. Democratic Republic of Timor-Leste. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1067. World Health Organization. Joint external evaluation of IHR core capacities of the Democratic republic of Timor-Leste, Mission report: 19-23 November 2018. No. WHO/WHE/CPI/2019.56. Geneva: World Health Organization; 2019.

1068. Ministry of Health Timor-Leste. National Health Sector Strategic Plan 2011-2030. Timor-Leste; 2011.

1069. Democratic Republic of Timor-Leste. System Of Epidemiological Surveillance Decree Law 09/2005. Timor-Leste; 2004.

1070. Republic of Togo. Ministry Of Health, Public Hygiene And Universal Access To Healthcare [Internet]. 2023 [cited 2023 Jan 21].

1071. World Health Organization. Évaluation externe conjointe des principales capacités RSI de la République Togolaise Rapport de mission: 16-20 avril 2018 [Joint external evaluation of the RSI core capacities of the Togolese Republic, Mission report: April 16-20, 2018]. No. WHO/WHE/CPI/REP/2018.31. Geneva: World Health Organization; 2018.

1072. Republic of Togo. Loi n° 2009-007 portant Code de la santé de la République togolaise. [Law No. 2009-007 on the Health Code of the Togolese Republic.]. Togo; 2009.

1073. Kingdom of Tonga. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1074. Tonga EWARS. Weekly Epidemiological Bulletin Early Warning Alert and Response System (EWARS) Tonga W11 2019 [Internet]. 2019 [cited 2022 Aug 20].

1075. Kingdom of Tonga. Public Health Act Chapter 28.30 2016 Revised Edition [Internet]. 2016 [cited 2022 Aug 20].

1076. Republic of Trinidad and Tobago. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1077. Republic of Trinidad and Tobago. Quarantine Act Chapter 28:05 Act 19 of 1944; Amended by 31 of 1978. 2016.

1078. Republic of Tunisia. Ministry of Public Health [Internet]. 2022 [cited 2022 Aug 21].

1079. World Health Organization. Joint External Evaluation of IHR Core Capacities of the Republic of Tunisia, Mission report: 28 November to 2 December 2016. No. WHO/WHE/CPI/REP/2017.45. Geneva: World Health Organization; 2017.

1080. Republic of Tunisia. Order of the Minister of Health fixing the list of communicable diseases subject to compulsory notification; amended 19 August 2020 [Internet]. 2015 [cited 2023 Jan 21].

1081. Republic of Turkey. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1082. Republic of Turkey. Bulaşici Hastaliklar Sürveyans Ve Kontrol Esaslari Yönetmeliği [Communicable Diseases Surveillance And Control Principles Regulation]. Turkey; 2007.

1083. Ministry of Health Turkey. Sağlik İstatistikleri Yilliği 2018 [Health Statistics Annual 2018]. Turkey; 2019.

1084. Republic of Turkey. List Of Communicable Diseases Based On Notification. No. RG-22/4/2020-31107 [Internet]. 2020 [cited 2022 Aug 21].

1085. Turkmenistan. Ministry of Health and Medical Industry [Internet]. 2023 [cited 2023 Jan 21].

1086. World Health Organization. Joint external evaluation of IHR core capacities of the Turkmenistan, Mission report: June 2016. No. WHO/WHE/CPI/2017.29. Geneva: World Health Organization; 2017.

1087. Government of Turkmenistan. Ýokanç keselleriň öňüni almak hakynda [The prevention of infectious diseases] No. 1, Article 11 [Internet]. Turkmenistan; 2021 [cited 2023 Jan 21].

1088. Tuvalu. Minister of Health, Social Welfare and Gender Affairs [Internet]. 2022 [cited 2022 Aug 22].

1089. Tuvalu. Public Health Regulations; 2008 Revised Edition CAP. 28.36.1. 1926 [cited 2022 Aug 22].

1090. Republic of Uganda. Ministry of Health [Internet]. 2023 [cited 2023 Jan 23].

1091. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Uganda, Mission report: June 26-30, 2017. No. WHO/WHE/CPI/REP/2017.49. Geneva: World Health Organization; 2017.

1092. Ministry of Health Republic of Uganda. Weekly Epidemiological Surveillance Brief, Week 18, 1 -7 May, 2022 [Internet]. 2022 [cited 2023 Jan 20].

1093. Republic of Uganda. Public Health Act 1935; Public Health (Control of COVID-19) Rules, 2020 [Internet]. 2020 [cited 2022 Aug 22].

1094. Ministry of Health Republic of Uganda. National Technical Guidelines for Integrated Disease Surveillance and Response [Internet]. Uganda; 2021 [cited 2022 Aug 22].

1095. Ukraine. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1096. Public Health Center of the Ministry of Health of Ukraine. Infectious morbidity of the population of Ukraine for November 2021 — November 2022 [Internet]. 2022 [cited 2023 Jan 23].

1097. Government of Ukraine. On Protection of Population against Infectious Diseases [Internet]. Ukraine; 2000 [cited 2022 Aug 22].

1098. United Arab Emirates. Ministry of Health and Prevention [Internet]. 2023 [cited 2023 Jan 20].

1099. World Health Organization. Joint external evaluation of IHR core capacities of the United Aram Emirates, Mission report: 19-23 March, 2017. No. WHO/WHE/CPI/REP/2017.57. Geneva: World Health Organization; 2017.

1100. Ministry of Health and Prevention United Arab Emirates. Open data of the Ministry of Health and Prevention [Internet]. 2022 [cited 2023 Jan 20].

1101. United Arab Emirates. Federal Law No. (13) of 2020 on Public Health [Internet]. United Arab Emirates: Ministry of Health and Prevention; 2020 [cited 2023 Jan 21].

1102. United Arab Emirates. Federal Law No. (14) of 2014 Concerning the Prevention of Communicable Diseases [Internet]. United Arab Emirates: Ministry of Health and Prevention; 2014 [cited 2023 Jan 21].

1103. United Kingdom. Department of Health & Social Care [Internet]. 2023 [cited 2023 Jan 22].

1104. UK Health Security Agency. Statutory Notifications Of Infectious Diseases In England And Wales Week 2022/51 [Internet]. 2023 [cited 2023 Jan 22].

1105. United Kingdom. Public Health (Control of Disease) Act 1984; last amendment 25 March 2022. United Kingdom; 1984.

1106. UK Health Security Agency. Guidance Notifiable diseases and causative organisms: how to report [Internet]. 2022 [cited 2023 Jan 22].

1107. United Kingdom. The Health Protection (Notification) Regulations 2010. United Kingdom; 2022.

1108. UK Health Security Agency. Infectious disease surveillance and monitoring for animal and human health: summary April to October 2022 [Internet]. 2023 [cited 2023 Jan 22].

1109. UK Health Security Agency. Syndromic surveillance: systems and analyses [Internet]. 2023 [cited 2023 Jan 22].

1110. Brownlie, J., Peckham, C., Waage, J., Woolhouse, M., Lyall, C., Meagher, L., Tait, J., Baylis, M. and Nicoll, A. Foresight. Infectious Diseases: preparing for the future. Office of Science and Innovation, London; 2006.

1111. UK Health Security Agency. Laboratory reporting to UKHSA: A guide for diagnostic laboratories [Internet]. 2022 [cited 2023 Jan 22].

1112. United States Department of Health & Human Services. Centers for Disease Control and Prevention [Internet]. 2023 [cited 2023 Jan 22].

1113. World Health Organization. Joint external evaluation of IHR core capacities of the United States of America, Mission report: June 2016. No. WHO/WHE/CPI/2017.13. Geneva: World Health Organization; 2017.

1114. United States Department of Health & Human Services. National Notifiable Diseases Surveillance System [Internet]. Centers for Disease Control and Prevention. 2023 [cited 2023 Jan 22].

1115. Centers for Disease Control and Prevention. 2023 National Notifiable Conditions. National Notifiable Diseases Surveillance System [Internet]. 2023 [cited 2023 Jan 22].

1116. United States Department of Health & Human Services. National Notifiable Diseases: Infectious Weekly Tables 2022 Week 52 [Internet]. 2023 [cited 2023 Jan 22].

1117. Government of the United States of America. Public Health Service Act; As Amended Through P.L. 117–241, Enacted December 20, 2022. United States; 2022.

1118. United States Department of Health and Human Services. Public Health Emergency Preparedness and Response Capabilities: National Standards for State, Local, Tribal, and Territorial Public Health. Centers for Disease Control and Prevention. 2018 [cited 2023 Jan 22].

1119. Republic of Uruguay. Ministry of Public Health [Internet]. 2023 [cited 2023 Jan 22].

1120. Oriental Republic of Uruguay. Diseases and events year 2011 [Internet]. 2013 [cited 2022 Aug 25].

1121. Oriental Republic of Uruguay. Decree 41/012. Uruguay; 2023.

1122. Oriental Republic of Uruguay. National Code of Mandatory Notification Diseases and Events [Internet]. 2018 [cited 2023 Jan 22].

1123. Republic of Uzbekistan. Ministry of Health [Internet]. 2023 [cited 2023 Jan 21].

1124. Republic of Uzbekistan. Law State Sanitary Supervision N 657-XII. Uzbekistan; 1992.

1125. Republic of Uzbekistan. On Additional Measures To Prevent The Spread Of Infectious Diseases In The Republic Of Uzbekistan [Internet]. 2017 [cited 2023 Jan 22].

1126. Republic of Vanuatu. Ministry of Health [Internet]. 2022 [cited 2023 Jan 22].

1127. Republic of Vanuatu. Public Health Act. Vanuatu; 2006.

1128. Bolivarian Republic of Venezuela. Ministry of Popular Power for Health [Internet]. 2022 [cited 2022 Aug 26].

1129. Bolivarian Republic of Venezuela. National Institute of Hygiene Rafael Rangel [Internet]. 2022 [cited 2022 Aug 26].

1130. Bolivarian Republic of Venezuela. Ley Orgánica de Salud [Organic Health Law] Nº 36.579/1998. Venezuela; 1998.

1131. Moronta G., Briceño E., Merino-Alado R., Ávila R., Campos Y., Lahoud K., Mendoza A., Salaverria A. Uso de la ficha de vigilancia epidemiológica por profesionales de la

salud en la Universidad Central de Venezuela [Use of the epidemiological surveillance record by healthcare professionals at the Central University of Venezuela]. Odous Científica. 2020 Dec [cited 2023 Jan 22];21(2):151–60.

1132. Socialist Republic of Viet Nam. Ministry of Health [Internet]. 2023 [cited 2023 Jan 22].

1133. World Health Organization. Joint external evaluation of IHR core capacities of Viet Nam, Mission report: 28 October - 4 November 2016. No. WHO/WHE/CPI/2017.21. Geneva: World Health Organization; 2017.

1134. Socialist Republic of Viet Nam. Viện Vệ Sinh Dịch Tễ Trung Ương [National Institute Of Hygiene And Epidemiology] [Internet]. 2023 [cited 2023 Jan 22].

1135. Socialist Republic of Viet Nam. On prevention and control of infectious diseases (No. 03/2007/QH12). Vietnam; 2007.

1136. Republic of Yemen. Ministry of Public Health and Population [Internet]. 2023 [cited 2023 Jan 22].

1137. Ministry of Public Health and Population Republic of Yemen. National Primary Health Care Strategy [Internet]. 2019 [cited 2022 Aug 28].

1138. Republic of Yemen. Regarding the regulations of the Ministry of Public Health and Population Republican Decree No. (76) of 2004 AD. Yemen; 2004.

1139. Government of Yemen. Yemen: Weekly Epidemiological Bulletin: Volume 09, issue 14, Epi week 14, (5-11 April, 2021) [Internet]. 2021 [cited 2023 Jan 22].

1140. Dureab F, Ahmed K, Beiersmann C, Standley CJ, Alwaleedi A, Jahn A. Assessment of electronic disease early warning system for improved disease surveillance and outbreak response in Yemen. BMC Public Health. 2020;20(1):1–11.

1141. Republic of Zambia. Ministry of Health [Internet]. 2023 [cited 2023 Jan 22].

1142. World Health Organization. Joint external evaluation of IHR core capacities of the Republic of Zambia, Mission report: 7-11 August 2017. No. WHO/WHE/CPI/REP/2017.50. Geneva: World Health Organization; 2017.

1143. Republic of Zambia. Zambia National Public Health Institute [Internet]. 2023 [cited 2023 Jan 22].

1144. Republic of Zambia. Annual Health Statistical Report 2017-2020. Zambia; 2021.

1145. Republic of Zambia. Public Health Act. Zambia; 1930.

1146. Republic of Zambia. The Public Health (Notifiable Infectious Disease) (Declaration) Notice. Zambia; 2020.

1147. Government of Zimbabwe. Ministry of Health and Child Care [Internet]. 2022 [cited 2022 Aug 31].

1148. World Health Organization. Joint external evaluation of IHR core capacities of the

Republic of Zimbabwe, Mission report: 19-23 February 2018. No. WHO/WHE/CPI/REP/2018.24. Geneva: World Health Organization; 2018.

1149. Republic of Zimbabwe. Public Health Act. Zimbabwe; 2018.

1150. World Bank. Population, total - United Kingdom [Internet]. The World Bank Group. 2020 [cited 2023 May 10].

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Appendix A: Chapter Two - Search Strategy

A search strategy was developed based on previously described methods and guidelines as adapted to government documents and other public health publications.[118,287] As recommended by Adams et al. (2016), a variety of search methods were deployed to ensure adequate information capture.[116] This is important for evidence synthesis, as the search strategy will determine the sensitivity and specificity of the returned results. While time intensive, increasing the search area to a wide range of resources for each country allows for robust data collection.

The adapted search strategy was informed by the methodology utilised by the Economist Impact for the 2021 version of the Global Health Security Index (GHSI).[287] This included ensuring all of the same official government sources (eg. Ministry of Health websites) were examined for each country. However, the aim of this study differed from the questions included in the 2021 GHSI, so different searches for this research were specifically developed for this paper and outlined below. Methodologies used in previous publications were also used to inform our strategy and adapted to our research question.[114–116] The relevant recommendations for best practice from a published Preferred Reporting Items for Systematic reviews and Meta-Analyses literature search extension (PRISMA-S) paper were included.[118]

A summary of the search strategy is included in the main paper in Figure 2.1. A checklist adapted for this study is in Table S1.[118] It should be noted that this paper is not a systematic review or meta-analysis and this checklist should be read as applicable to the search methodology used for this research.

Table S1. Checklist for the search strategy used for this current paper, with locations in the appendix of the checklist items included. This checklist is adapted from PRISMA-S which was designed for systematic reviews and meta-analyses, not grey literature searches, so is presented as an adaptation to this type of methodology. PRISMA-S: Preferred Reporting Items for Systematic reviews and Meta-Analyses literature search extension.[118]

Section/Topic	Item #	Checklist Item	Location
INFORMATION SOURCES AND METHODS			
Database name	1	Name each individual database searched, stating the platform for each.	Appendix A
Multi-database searching	2	Appendix A	Appendix A
Study registries	3	List any study registries searched.	N/A
Online resources and browsing	4	Describe any online or print source purposefully searched or browsed (e.g., tables of contents, print conference proceedings, websites), and how this was done.	Appendix A
Citation searching	5	Indicate whether cited references or citing references were examined, and describe any methods used for locating cited/citing references (e.g., browsing reference lists, using a citation index, setting up email alerts for references citing included studies).	Appendix A
Contacts	6	Indicate whether additional studies or data were sought by contacting authors, experts, manufacturers, or others.	Appendix A
Other methods	7	Describe any additional information sources or search methods used.	Appendix A
SEARCH STRATEGIES			
Full search strategies	8	Include the search strategies for each database and information source, copied and pasted exactly as run.	Appendix A
Limits and restrictions	9	Specify that no limits were used, or describe any limits or restrictions applied to a search (e.g., date or time period, language, study design) and provide justification for their use.	Appendix A
Search filters	10	Indicate whether published search filters were used (as originally designed or modified), and if so, cite the filter(s) used.	N/A
Prior work	11	Indicate when search strategies from other literature reviews were adapted or reused for a substantive part or all of the search, citing the previous review(s).	Appendix A
Updates	12	Report the methods used to update the search(es) (e.g., rerunning searches, email alerts).	Appendix A
Dates of searches	13	For each search strategy, provide the date when the last search occurred.	Appendix A
PEER REVIEW			
Peer review	14	Describe any search peer review process.	N/A
MANAGING RECORDS			
Total Records	15	Document the total number of records identified from each database and other information sources.	Appendix B
Deduplication	16	Describe the processes and any software used to deduplicate records from multiple database searches and other information sources.	N/A

A.1 Resources Examined

The following databases and resources were examined for each of the 195 Countries:

1. The Global Health Security Index (GHSI), including the 2021 Full Report,[48] and the 195 individual Country Score Justification Summaries, which include all the data explanations and referenced sources for the Index. Citation searching through reference list browsing was undertaken to generate a list of all study-relevant and country-specific government websites and published national resources and documents.

2. All 104 World Health Organisation (WHO) Joint External Evaluations completed and published as of 31 December 2022. These were conducted at the national level with participating Member States using a methodology previously described.[288] All Joint External Evaluations underwent citation searching through reference list browsing to generate a list of all referenced public health legislation, nationally notifiable disease lists and published country-specific guidelines. These reports were also examined for evidence of how the country's infectious disease surveillance system functions, including the reported number of diseases under surveillance and the frequency of internal national data analysis.

3. All official Ministry of Health government websites, or their equivalent, as available and accessible for the 195 included countries.

4. All official national Public Health and Epidemiology websites and disease surveillance data publishing platforms, or their equivalent, as available and accessible for the 195 included countries.

5. All official Ministry of Justice government websites and online national legislative databases, or their equivalent, as available and accessible for the 195 included countries.

6. All National Reference Laboratory websites, or their equivalent, as available and accessible for the 195 included countries.

7. National law repositories from the World Legal Information Institute (WorldLII),[289] the Verification Research, Training and Information Centre (VERTIC),[290] and the NATLEX legislation database.[291]

8. Country-specific pages in regional infectious disease surveillance networks, including the Pacific Public Health Surveillance Network (PPHSN),[292] the Pan American Health Organization (PAHO),[293] and the Caribbean Public Health Agency (CARPHA).[294] Aggregate country data were also explored at the European Centre for Disease Prevention and Control (ECDC),[295] and the World Health Organisation.[296] Citation search was undertaken through reference list browsing of these resources for each country. Only national resources published by the country itself, whether solo or in collaboration with another entity, were included in the dataset.

9. Ovid with simultaneous searches in Embase, Medline and the Global Health databases. A separate search was performed on Google Scholar. Given that published literature on national disease surveillance could be inconsistent with and out-of-date when compared with recently updated government resources, peer-reviewed publications were only used when no equivalent government resource existed.

10. Google Search was utilised as described below to assist with finding documents and resources with the search-within-site function deployed.

Data collection took place between July and December 2022. All sources were re-examined in January 2023 for any new evidence publications, with updates made to the dataset as required. A data date cut-off of 31 December 2022 was chosen to standardise comparison. Data validation was carried out by the research team. Because our aim was to discover information on novel disease considerations specifically in resources that were publicly available, national public health authorities were not contacted for direct internal information.

A.2 Main Search Strategy and Terms

The country-specific government websites and resources were the primary source of the data included in the dataset. The search strategy for these is described below, and was repeated for each of the 195 countries using the resources obtained from the locations listed above. Because government sources often were more up-to-date than peer-reviewed publications, resources published by government were used over that of journal articles. For example, some nationally notifiable disease lists are published in peer-reviewed literature, but these were often not the current active list, and thus the official government resource was favoured.

The search strategy performed for each of the data fields is outlined below and was carried out on a country-by-country basis in the listed resources. Search terms were chosen to be both specific to limit results to novel disease surveillance and sensitive enough to capture documents with relevant information.

Novel disease consideration search terms: "novel disease" or "new disease" or "unknown disease" or "unexplained disease" or "disease of unknown cause" or "disease of unknown aetiology" or "disease of unknown etiology" or "novel pathogen" or "new pathogen" or "unknown pathogen" or "unidentified pathogen“ or “events of public health concern” or “unusual event” or “unexplained event” or “unusual death” or “unexplained death”

Resource-specific information included in the dataset with explanation and search terms, where relevant:

1. Availability of national public health law (Yes/No)
    a. **Yes**: publicly available and accessible national level (including Laws, Acts, Orders, Decrees and Regulations) related to one or more of the following: Public Health, Medicine, Health, Infectious Diseases, Epidemics, Notification, or Disease Surveillance
    b. **No**: Such national legislation does not exist or is not publicly available and accessible online
2. Novel pathogen surveillance in national public health law (Yes/No)

    a. **Yes**: publicly available and accessible law contains references one or more of the novel disease consideration search terms and the context indicates novel disease consideration, and not just limited to recently discovered new diseases or novel influenza subtypes exclusively
    b. **No**: The publicly available and accessible law does not reference these terms or the context makes it clear it is not referring to novel disease consideration, or the law is unavailable
3. Availability of a nationally notifiable disease list (Yes/Number only/No)
    a. **Yes**: publicly available and accessible nationally notifiable disease list published in full found using the following terms: nationally notifiable disease list, notifiable conditions, diseases with mandatory reporting, infectious disease list, surveillance list
    b. **Number only**: No nationally notifiable disease list is accessible but the total number of diseases under surveillance is published and available
    c. **No**: No nationally notifiable disease list or the number of diseases under surveillance is publicly available and accessible online
4. Total number of infectious diseases under national surveillance (Number, NA)
    a. **Number:** Reported if either the nationally notifiable disease list was available or the number of diseases under surveillance had been published
    b. **NA**: No nationally notifiable disease list or the number of diseases under surveillance is publicly available and accessible online
5. Location of national notifiable disease list (Legislation, Publication, Website, Report, Article, NA). In cases where the disease list was present in more than one location, the most recent list was used, or if they were equivalent, the following order was used:
    a. **Legislation**: The national notifiable disease list is published in full within national legislation
    b. **Publication**: A national government document, including national guidelines, an overview of the national disease surveillance system, or a stand-alone government-published list
    c. **Website:** Official government website with a specific webpage dedicated to the national notifiable disease list
    d. **Report**: Published epidemiological data report which specifies that the included diseases are notifiable conditions. Data reports generated solely by international institutions or lower than the country level are not included; data reports generated in collaboration with the national government were permissible.
    e. **Article**: The national notifiable disease list is published within a journal article
    f. **NA**: No nationally notifiable disease list or the number of diseases under surveillance is publicly available and accessible online
6. Novel pathogen surveillance in the nationally notifiable disease list (Yes/No)
    a. **Yes**: The nationally notifiable disease list contains one or more of novel disease consideration search terms and the context indicates novel disease consideration, and not just limited to recently discovered new diseases or novel influenza subtypes exclusively
    b. **No**: The nationally notifiable disease list does not contain one or more of the search terms, or no nationally notifiable disease list or the number of diseases under surveillance is publicly available and accessible online
7. Availability of health professional guidance on novel pathogen surveillance (Yes/No)

   a. **Yes**: An official government guidance document (surveillance system guidelines, notifiable disease reporting guidelines, public health strategy documents) exists for health professionals (doctors, community health workers or equivalent) which references one or more of the novel disease consideration search terms and the context indicates novel disease consideration, and not just limited to recently discovered new diseases or novel influenza subtypes exclusively
   b. **No**: There is no publicly available and accessible health professional guidance document which includes the novel disease consideration search terms or relevant context
8. Type of health professional guidance (guide and form, guide only, form only, mention only, NA)
   a. **Guide and form**: Both written guidelines and a reporting form for novel diseases are publicly available and accessible
   b. **Guide only**: Only written guidelines on novel diseases are publicly available and accessible
   c. **Form only**: Only a reporting form for novel diseases is publicly available and accessible
   d. **Mention only**: Novel diseases (or equivalent terms) are briefly mentioned in a single sentence or section but no further details are provided
   e. **NA**: There is no publicly available and accessible health professional guidance document which includes the novel disease consideration search terms or relevant context
9. Availability of laboratory guidance on novel pathogen surveillance (Yes/No)
   a. **Yes**: An official government guidance document (reference laboratory guidelines, disease testing strategy, public health laboratory protocols) exists for laboratories (public health reference laboratory, national laboratory or sub-national general laboratory guidance) which references one or more of the novel disease consideration search terms and the context indicates novel disease consideration, and not just limited to recently discovered new diseases or novel influenza subtypes exclusively
   b. **No**: There is no publicly available and accessible laboratory guidance document which includes the novel disease consideration search terms or relevant context
10. Type of laboratory guidance (guide and protocol, guide only, protocol only, mention only, NA)
   a. **Guide and protocol**: Both written guidelines and a protocol for testing for novel diseases are publicly available and accessible
   b. **Guide only**: Only written guidelines on novel diseases for laboratories are publicly available and accessible
   c. **Form only**: Only a protocol for novel diseases for laboratories is publicly available and accessible
   d. **Mention only**: Novel diseases (or equivalent terms) are briefly mentioned but no details are provided in available laboratory guidance
   e. **NA**: There is no publicly available and accessible laboratory guidance document which includes the novel disease consideration search terms or relevant context
11. Highest frequency of surveillance data analysis (Frequency)
   a. Frequency: Stated frequency of analysis of collected infectious disease surveillance system data at the national level. If there are different frequencies

of data analysis in available resources depending on the disease in question, the highest frequency reported is included

12. Published national infectious disease surveillance data available (Yes/No)
    a. **Yes**: Published national infectious disease surveillance data on some or all nationally notifiable diseases is publicly available and accessible online
    b. **No**: There is no published national infectious disease surveillance data online or the only data available is for a specific disease or disease group only (eg. influenza)
13. Date of last published national infectious disease surveillance data
    a. **Date**: Day-month-year of the last data recorded (publication data is in the references) with study cut-off date of 31-Dec-2022
    b. **NA**: There is no published national infectious disease surveillance data available
14. Stated frequency of published national infectious disease surveillance data (Frequency)
    a. **Frequency**: The specified reporting frequency of the last published national infectious disease surveillance data regardless of when the last data were published
    b. **NA**: There is no published national infectious disease surveillance data available

Ministry of Health and other government websites had variable internal indexing and search function capabilities. To increase the sensitivity of the search during data collection, the search-within-site function on Google was deployed in addition to on-site searches. Non-English websites and documents were translated using Google Translate and the on-site Chrome Google Translate extension.[297] Search terms were converted to the national language for resource acquisition. Previously, a 2012 study showed mixed evidence for Google Translate's utility in academic research.[298] However, since then machine translation has improved drastically and a 2019 study found Google Translate robust and accurate enough to recommend it for use in systematic reviews.[299] Therefore, it was deemed appropriate for this current study to deploy as without it significant language-bias would be introduced into the returned search results.

A.3 Database Search Strategy

A simultaneous search of Embase, Medline and the Global Health Searches were conducted on the Ovid online platform from database inception to 31 December 2022. A secondary search was conducted on Google Scholar. The primary aims of the database search were to find documents and resources not uncovered in the country-specific resource locations, such as national notifiable disease lists or background infectious disease surveillance system information. Articles on national disease surveillance also underwent citation searching for government documents referenced. The following searches were conducted with the specific country name included as indicated.

1. **Embase, Medline and Global Health via the Ovid online platform search terms:** ((“COUNTRY NAME”) and ("disease surveillance" or "indicator surveillance" or "indicator-based surveillance" or "public health surveillance")).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, cw, bt, nm, ox, px, rx, ui, sy, ux, mx]

2. **Embase, Medline and Global Health via the Ovid online platform search terms:** ((“COUNTRY NAME”) and ("notifiable disease list" or "notifiable disease*" or "diseases under surveillance" or "mandatory notif*")).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, cw, bt, nm, ox, px, rx, ui, sy, ux, mx]
3. **Google Scholar search terms**: (“COUNTRY NAME” and "disease surveillance") or (“COUNTRY NAME” and "indicator surveillance") or (“COUNTRY NAME” and "indicator-based surveillance") or (“COUNTRY NAME” and “public health surveillance")
4. **Google Scholar search terms**: (“COUNTRY NAME” and "notifiable disease list") or (“COUNTRY NAME” and "notifiable disease") or (“COUNTRY NAME” and "diseases under surveillance") or (“COUNTRY NAME” and “mandatory notification")

A.4 Comparison Data

In addition, the following data was gathered and collated for each of the 195 countries, with reference sources:

1. Has the country completed and published a WHO Joint External Evaluation?[300]
    a. Yes/No
2. Has the country reported having one or more cases of Nipah virus?[301]
    a. Yes/No
3. Has the country reported having one or more cases of MERS?[91]
    a. Yes/No
4. Has the country reported having one or more cases of SARS-CoV-1?[302]
    a. Yes/No
5. How many outbreaks has the country experienced between January 1996 - March 2022?[121]
    a. Number of reported outbreaks
6. What is the country's World Bank income group based on the 2021 gross national income (GNI)?[119]
    a. High Income/Upper middle income/Lower middle income/Low income
7. What is the country's World Bank geographical region?[119]
    a. East Asia and Pacific/Europe and Central Asia/Latin America & the Caribbean/Middle East and North Africa/North America/South Asia/Sub-Saharan Africa
8. What is the country's 2019 health expenditure, as percent of GDP?[303]
    a. Percentage
9. What is the country's 2019 health expenditure per capita, in current USD?[120]
    a. $USD
10. What is the country's domestic general government health expenditure, as a percent of GDP in 2019?[304]
    a. Percentage
11. What is the country's total population in 2021?[305]
    a. Number of people
12. What is the country's GDP per capita in 2021, in current USD?[306]
    a. $USD
13. What is the country's GDP, in current USD in 2021?[307]

    a. $USD
14. Has this WHO African Region country adapted the Integrated Disease Surveillance and Response (IDSR) second edition as of December 2017?[308]
    a. Yes/No
15. For this WHO African Region country, how many districts have had IDSR training between 2015 and 2017?[308]
    a. Greater than 90%/50 - 89%/Less than 50%
16. What is the country's Human Development Index score for 2021?[309]
    a. Number [0-1]
17. Which WHO region does this country belong?[111]
    a. AFRO/AMRO/EMRO/EURO/SEARO/WPRO
18. 2021 International Health Regulations (2005) (IHR[2005]) States Parties Self-Assessment Annual Reporting (SPAR) dataset[111]
    a. 15 capacities and 35 indicators
19. Joint External Evaluation published as of 31 December 2022[300]
    a. 19 capacities
20. 2021 GHSI dataset[48]
    a. Six categories, 37 indicators, and 171 individual questions

A.5 Specific SPAR and GHSI Indicators

Two questions specific to novel diseases were also compared from the 2021 SPAR and GHSI datasets, with the full wording of each indicator as below:

1. 2021 SPAR C4.4. Laboratory testing capacity modalities - Level 5[310]
    a. **Yes/No**: Laboratory system can perform in all capacities including access to whole genome sequencing; identification of unknown and high consequence pathogens and has access to viral culture. Laboratory networks configured to support all diagnostic services that are integrated are sustainable, with maximum population coverage, and exercised, reviewed, evaluated and updated on a regular basis as applicable
    b. Notes:
        i. Refers to laboratory test capacities that are available within the country (including research laboratories and private laboratories) to support surveillance and response; or that are available through referral mechanisms to designated central or international reference laboratories (e.g., WHO collaborating centres).
        ii. Priority diseases include, epidemic prone diseases, diseases earmarked for eradication/elimination and diseases of public health importance.
        iii. Access to whole genome sequencing may be through international collaboration including WHO collaborating centres.
        iv. This may include whole genomic sequencing and access to whole genome sequencing may be through international collaboration including WHO collaborating centres.
        v. Between the human, animal and environmental health sectors.

2. 2021 GHSI Category 2: Early Detection and Reporting for Epidemics of Potential International Concern - 2.1 Laboratory systems strength and quality - 2.1.1 Laboratory capacity for detecting priority diseases - GHSI 2.1.1b[287]
    a. **All/Some/No**: Is there a national plan, strategy, or similar document for conducting testing during a public health emergency, which includes considerations for testing for novel pathogens, scaling capacity, and defining goals for testing?
        i. **All**: Yes, there is evidence of a plan, and it includes considerations for testing for novel pathogens, scaling capacity, and defining goals for testing
        ii. **Some:** Yes, there is evidence of a plan, but there is insufficient evidence that it includes considerations for testing for novel pathogens, scaling capacity, and defining goals for testing
        iii. **No:** No evidence of a plan

In addition, a comparison was made with the data gathered by the 2021 GHSI on event-based surveillance, with the full wording below:

3. 2021 GHSI Category 2: Early Detection and Reporting for Epidemics of Potential International Concern - 2.3 Real-time surveillance and reporting - 2.3.1 Indicator and event-based surveillance and reporting systems - 2.3.1a[287]
    a. **Yes daily/Yes not daily/No:** Is there evidence that the country is conducting ongoing event-based surveillance and analysis for infectious disease?
        i. **Yes daily:** Yes, there is evidence of ongoing event-based surveillance and evidence that the data is being analyzed on a daily basis
        ii. **Yes not daily:** Yes, there is evidence of ongoing event-based surveillance, but no evidence that the data is being analyzed on a daily basis
        iii. **No** = No

Appendix B: Chapter Two - Data Sources

B.1 Dataset

All data generated and used for Chapter Two are available for download through the Open Science Framework (OSF) platform, along with all code used to generate the results, tables and figures. In addition to the data, OSF contains the data dictionary, sources, and code used to generate all tables and figures in Chapter Two and Appendix C. All references for this Appendix are also available on OSF in table format.

B.2 Country References

The references for data fields included in the dataset are specified below for each country.

1. **Afghanistan**: The data obtained included information from the Ministry of Public Health,[311] the country's published WHO Joint External Evaluation report,[312] the National Health Policy,[313] and the *Public Health Law*.[314] The latest National Disease Surveillance and Response report included the number of diseases under surveillance and the nationally notifiable disease list.[315]

2. **Albania**: The data obtained included information from the Ministry of Health and Social Protection,[316] the country's published WHO Joint External Evaluation report,[317] the *Public Health Law*,[318] the law entitled *For the prevention and fight against infections and infectious diseases*,[319] and the national COVID-19 Action Plan.[320] National disease surveillance data were available and included information on a subset of the nationally notifiable diseases.[321] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[317] Under national law, new infectious diseases and threats of unknown origin are subject to mandatory reporting.[319]

3. **Algeria**: The data obtained included information from the Ministry of Health,[322] a published article detailing the national public health surveillance system and the nationally notifiable disease list,[323] and the *Law on the Protection and Promotion of Health.*[324] No nationally published disease surveillance data were available. A WHO Joint External Evaluation was completed in March 2022, but no report has been made available as of 31 December 2022.[300]

4. **Andorra**: The data obtained included information from the Ministry of Health, Social Affairs and Employment,[325] the National Health Plan 2020,[326] the *General Health Law,*[327] and the list of nationally notifiable diseases included in annually published disease surveillance data.[328] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

5. **Angola:** The data obtained included information from the Ministry of Health,[329] the country's published WHO Joint External Evaluation report,[330] the National Health Development Plan,[331] the National Contingency Plan for the Control of the

Coronavirus Disease Epidemic 2019,[332] and the *Organic Statute of the Ministry of Health.*[333] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible.

6. **Antigua and Barbuda:** The data obtained included information from the Ministry of Health,[334] the *Public Health Act* which includes the nationally notifiable disease list,[335] and the national COVID-19 Guidelines.[336] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

7. **Argentina:** The data obtained included information from the Ministry of Health,[337] the National Administration of Laboratories and Health Institutes,[338] the *Public Health Law*,[339] amended in 2022 with a *Ministry of Health Resolution*,[340] a manual on mandatory disease reporting,[341] and National Health Surveillance System Guidelines which contains the nationally notifiable disease list.[342] The Ministry of Health provides guidelines for healthcare professionals on reporting diseases of unknown aetiology and provides a specific reporting form.[149] There is a separate guideline for acute respiratory infections of possible viral origin.[343] This includes laboratory guidance mandating reporting of negative laboratory test results for severe acute respiratory infections of unknown aetiology. The latest published national disease surveillance data were available.[344] Argentina completed a WHO Joint External Evaluation in August 2019, but there has not been a published report as of 31 December 2022.[300]

8. **Armenia:** The data obtained included information from the Ministry of Health,[345] the National Center for Disease Control and Prevention,[346] the country's published WHO Joint External Evaluation report,[347] the Ministry of Health rules on epidemiological surveillance for infectious diseases,[348] and the Ministry of Health standards and rules.[349,350] The *Mechanisms of Cooperation And Coordination Procedures Of The National Coordinating Body And Interested Bodies In Questions Of International Health (Sanitary) Rules* mandates reporting of unusual diseases with an unknown cause.[351] No nationally published disease surveillance data is available.

9. **Australia:** The data obtained included information from the Department of Health and Aged Care,[352] the country's published WHO Joint External Evaluation report,[353] the National Notifiable Disease Surveillance System list,[354] the *Biosecurity Act 2015*,[355] and the National Notifiable Diseases Surveillance System.[356] The Emergency Response Plan for Communicable Disease Incidents of National Significance mentions the emergence of previously unknown diseases but does not provide detailed guidance.[357] The latest published nationally notifiable disease report was available.[358]

10. **Austria:** The data obtained included information from the Ministry of Health,[359] the *Epidemic Act 1950* with 2022 amendments,[360] and the latest quarterly published disease surveillance data, which includes the nationally notifiable disease list.[361] The national syndrome-based infectious disease surveillance activities are outlined in a published article.[362] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

11. **Azerbaijan:** The data obtained included information from the Ministry of Health,[363] a published article on the national infectious disease surveillance system,[364] the law *On Protection of the Health of the Population*,[365] and health professional guidelines.[366] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

12. **Bahamas:** The data obtained included information from the Ministry of Health,[367] published disease surveillance data,[368] and the *Health Services Health Rules*,[369] which includes the nationally notifiable disease list. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

13. **Bahrain:** The data obtained included information from the Ministry of Health,[370] the country's published WHO Joint External Evaluation report,[371] nationally published disease surveillance data with nationally notifiable disease list,[372] and the *Public Health Law*.[373]

14. **Bangladesh:** The data obtained included information from the Ministry of Health and Family Welfare,[374] the country's published WHO Joint External Evaluation report,[375] the National Public Health Laboratory Strategy for Infectious Diseases,[376] the Institute of Epidemiology, Disease Control & Research Outbreak report,[377] and the *Communicable Diseases (Prevention, Control and Eradication) Act* which includes the nationally notifiable disease list.[378] Published articles on the national disease surveillance system indicate unknown diseases are reported and investigated nationally.[52,379] No nationally published disease surveillance data is available.

15. **Barbados:** The data obtained included information from the Ministry of Health and Wellness,[380] the *Health Services (Communicable And Notifiable Diseases) Regulations* which includes the nationally notifiable disease list,[381] the *Emergency Management (Covid 19) Order*,[382] and the Caribbean Public Health Agency report.[383] No nationally published disease surveillance data is available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

16. **Belarus:** The data obtained included information from the Ministry of Health,[384] the *Emergency notification of infectious diseases* which includes the list of nationally notifiable diseases,[385] and the Republican Center for Hygiene, Epidemiology and Public Health.[386] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

17. **Belgium:** The data obtained included information from the Federal Public Service for Health,[387] the Sciensano Epistat website which provides surveillance data on notifiable diseases,[388] the country's published WHO Joint External Evaluation report,[389] and the nationally notifiable disease lists in Belgium's three regions.[390–392] Severe respiratory syndromes in the context of an emerging virus and unusual presentations of an infectious disease are included in the notifiable disease list.

18. **Belize:** The data obtained included information from the Ministry of Health and Wellness,[393] the *Public Health Act* which contains the nationally notifiable disease list,[394] and the Caribbean Public Health Agency report.[383] No nationally published disease surveillance data is available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

19. **Benin:** The data obtained included information from the Ministry of Health,[395] the country's published WHO Joint External Evaluation report,[396] and a published article on public health surveillance in Benin.[397] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[396]

20. **Bhutan:** The data obtained included information from the Ministry of Health,[398] the country's published WHO Joint External Evaluation report,[399] the Bhutan Pandemic Preparedness and Response Plan,[400] the latest published national disease surveillance data,[401] and the WHO Health System Review.[402] A publication on the National Early Warning, Alert and Response Surveillance (NEWARS) includes guidance on unknown disease outbreaks with mandatory reporting for any cluster of death involving two or more people at the same location and time.[157] There is a National Health Policy available but no relevant public health legislation, and this was confirmed also in the WHO Joint External Evaluation report.[399,403]

21. **Bolivia:** The data obtained included information from the Ministry of Health and Sports,[404] the latest published national disease surveillance data,[405] and the Ministry of Health *Supreme Decrees*.[406] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

22. **Bosnia and Herzegovina:** The data obtained included information from the Federal Ministry of Health,[407] the Epidemiology Service,[408] and the annual Report On Infectious Diseases.[409] The *Law On The Protection Of The Population Against Infectious Diseases* contains the nationally notifiable disease list and includes infectious diseases and epidemics of unknown origin, but not on the list itself.[410] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

23. **Botswana:** The data obtained included information from the Ministry of Health,[411] the country's published WHO Joint External Evaluation report,[412] the latest published national disease surveillance data,[413] the *Public Health Act*,[414] and the Integrated Health Service Plan.[415] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[412]

24. **Brazil:** The data obtained included information from the Ministry of Health,[416] the latest epidemiological bulletin,[417] the nationally notifiable disease list,[418] the Operation Plan of the National Focal Point for the International Health Regulations,[419] the Plan for Strengthening and Expansion of the National Hospital Epidemiological Surveillance Network,[420] and the *National List of Compulsory Notification of Diseases.*[421] The national notifiable disease system guidance includes reporting requirements on unusual conditions or deaths from disease of unknown origin, and specifies these are mandated to be notified in legislation and the notifiable disease list for unusual outbreaks involving at least two cases that are epidemiologically linked.[422] There is an outbreak reporting form,[423] with instruction sheet,[424] specifying unusual and unknown diseases should be reported. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

25. **Brunei:** The data obtained included information from the Ministry of Health,[425] the *Infectious Diseases Act* which includes the nationally notifiable disease list,[426] the country's published WHO Joint External Evaluation report,[427] and the Strategic Health Plan.[428] No nationally published disease surveillance data were available.

26. **Bulgaria:** The data obtained included information from the Ministry of Health,[429] the latest national epidemiology report,[430] the *Health Law* which includes new and unknown infectious diseases,[431] and an Overview Of Communicable Disease Surveillance System In Bulgaria with nationally notifiable disease list.[432] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

27. **Burkina Faso:** The data obtained included information from the Ministry of Health and Public Hygiene,[433] the country's published WHO Joint External Evaluation report,[434] disease surveillance system guidelines which contains the nationally notifiable disease list,[435] and an event-based surveillance implementation overview.[436]No nationally published disease surveillance data were available.

28. **Burundi:** The data obtained included information from the Ministry of Health,[437] the National Institute of Public Health,[438] and the *Public Health Code* which includes the nationally notifiable disease list.[439] No nationally published disease surveillance data were available.

29. **Cabo Verde:** The data obtained included information from the Ministry of Health,[440] the country's published WHO Joint External Evaluation report,[441] national health statistics with nationally notifiable disease list,[442] and the National Institute of Public Health.[443]

30. **Cambodia:** The data obtained included information from the Ministry of Health,[444] the country's published WHO Joint External Evaluation report,[445] the latest disease surveillance data report which includes the nationally notifiable disease list,[446] Public health legislation is pending enactment, and this was also confirmed in the WHO Joint External Evaluation report.[445]

31. **Cameroon:** The data obtained included information from the Ministry of Public Health,[447] the country's published WHO Joint External Evaluation report,[448] and the country's core health indicators publication with notifiable disease surveillance data.[449] No complete nationally notifiable disease list was publicly accessible. There is no relevant public health legislation available, and this was also confirmed in the WHO Joint External Evaluation report.[448]

32. **Canada:** The data obtained included information from the Public Health Agency,[450] the country's published WHO Joint External Evaluation report,[451] the nationally notifible disease list, [452] nationally notifiable disease surveillance data,[453] and the *Public Health Agency of Canada Act*.[454] The latest Canadian Public Health Laboratory Network Strategic Plan mentions the need for an all-hazards plan for unknown pathogens but does not provide further guidance.[455]

33. **Central African Republic:** The data obtained included information from the Ministry of Health and Population,[456] and the country's published WHO Joint

External Evaluation report.[457] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. There is no relevant public health legislation available, and this was also confirmed in the WHO Joint External Evaluation report.[457]

34. **Chad:** The data obtained included information from the Ministry of Health and National Solidarity,[458] the country's published WHO Joint External Evaluation report,[459] and the National Health Policy 2016-2030.[460] No nationally published disease surveillance data were available. The WHO Joint External Evaluation report specifies the diseases under surveillance.[459] No nationally published disease surveillance data were available. There is a National Health Policy available but no relevant public health legislation, and this was confirmed in the WHO Joint External Evaluation report.[459,460]

35. **Chile:** The data obtained included information from the Ministry of Health,[461] notifiable disease guidance with nationally notifiable disease list,[155] and the latest epidemiological bulletin.[462] The notifiable disease guidance mentions reporting diseases and outbreaks of unknown cause, including deaths due to an unidentified transmissible pathogen, but this is not included in the nationally notifiable disease list.[155] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

36. **China:** The data obtained included information from the Chinese Center for Disease Control and Prevention,[463] Ministry of Health guidance on reporting unknown pneumonia cases,[159] the National Emergency Response Plan for Public Health Emergencies,[464] published articles on the nationally notifiable disease system,[113,465] and the latest published national notifiable infectious disease surveillance data.[466] The *Emergency regulations for public health emergencies* requires reporting new infectious diseases and diseases of unknown cause.[158]

37. **Colombia:** The data obtained included information from the National Institute of Health,[467] the latest epidemiological bulletin,[468] the National Guidelines For Surveillance including the nationally notifiable disease list,[469] the collection of public health laws,[470] and the Surveillance Protocol for Acute Respiratory infections which includes those caused by unknown pathogens.[471] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

38. **Comoros:** The data obtained included information from the country's published WHO Joint External Evaluation report,[472] a published article on the Indian Ocean epidemic surveillance and alert management network SEGA, [473] and the *Public Health and Action Code*.[474] The Ministry of Health's online presence is limited to an official Facebook page.[475] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

39. **Congo (Brazzaville):** The data obtained included information from the Ministry of Health,[476] the country's published WHO Joint External Evaluation report,[477] and the

latest epidemiological bulletin.[478] No complete nationally notifiable disease list was publicly accessible. There is no relevant public health legislation available, and this was confirmed also in the WHO Joint External Evaluation report.[477]

40. **Congo (Democratic Republic):** The data obtained included information from the Ministry of Health,[479] the country's published WHO Joint External Evaluation report,[480] the National Institute of Biomedical Research,[481] and a published article on the disease surveillance system.[482] There is a National Health Development Plan,[483] but relevant public health legislation is still pending, as confirmed by the WHO Joint External Evaluation report.[480] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

41. **Cook Islands:** The data obtained included information from the Ministry of Health,[484] the *Public Health Act,*[485] and the *Public Health (Notifiable Conditions) Order* which contains the nationally notifiable disease list.[486] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

42. **Costa Rica:** The data obtained included information from the Ministry of Health,[487] the nationally notifiable disease list,[488] latest epidemiological bulletin,[489] and the *General Health Law.*[490] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

43. **Côte d'Ivoire:** The data obtained included information from the Ministry of Health, Public Hygiene and Universal Health,[491] the National Institute of Public Hygiene,[492] the *Creating the National Public Health Laboratory Decree*,[493] the country's published WHO Joint External Evaluation report,[494] and health sector policy survey.[495] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

44. **Croatia:** The data obtained included information from the Ministry of Health,[496] the law *On The Protection Of The Population Against Infectious Diseases* which includes the nationally notifiable disease list,[497] and the latest annual nationally published disease surveillance data.[498] Legislation includes reporting requirements for diseases of unknown origin, and unusual events that may indicate an epidemic of unknown cause.[497] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

45. **Cuba:** The data obtained included information from the Ministry of Health,[499] the latest Statistical Yearbook of Health,[500] the *Public Health Law*,[501] and published article on the national disease surveillance system.[502] No complete nationally notifiable disease list was publicly accessible. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

46. **Cyprus:** The data obtained included information from the Ministry of Health,[503] the *Quarantine Law* which includes the nationally notifiable disease list,[504] and the latest Ministry of Health Strategic Plan.[505] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

47. **Czech Republic:** The data obtained included information from the Ministry of Health,[506] the national pandemic plan,[507] the latest published disease surveillance data,[508] and the *Protection of Public Health Act* which includes provisions on new infectious diseases of unknown origin.[509] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

48. **Denmark:** The data obtained included information from the Ministry of the Interior and Health,[510] the Statens Serum Institute published notifiable disease data dashboard,[511] the *Proclamation on generally dangerous diseases*,[512] and *Order on doctors' notification of infectious diseases* which includes the nationally notifiable disease list.[513] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

49. **Djibouti:** The data obtained included information from the Ministry of Health,[514] the country's published WHO Joint External Evaluation report,[515] and the *Law No. 118 Relating to the powers and organization of the Ministry of Health*.[516] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[515] No nationally published disease surveillance data were available.

50. **Dominica:** The data obtained included information from the Ministry of Health,[517] the *Environmental Health Services (Communicable and Notifiable Diseases) Regulations* which contains the nationally notifiable disease list,[518] and the *Regulations* 2020 amendment.[519] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

51. **Dominican Republic:** The data obtained included information from the Ministry of Health and Social Assistance,[520] national hospital health plan,[521] the latest epidemiological bulletin,[522] and *Mandatory Report of Prioritized Diseases and Events*.[523] The Dominican Republic completed a WHO Joint External Evaluation in September 2019, but there has not been a published report as of 31 December 2022.[300]

52. **Ecuador:** The data obtained included information from the Ministry of Public Health,[524] the national surveillance system manual, the National Directorate of Epidemiological Surveillance,[525] and the latest national epidemiological bulletin.[526] The notifiable disease list and surveillance system manual includes unusual events and unknown diseases, with a guideline for health professionals on reporting and a

form.[527] In this document there is an algorithm protocol for laboratories for unusual respiratory infections but no detailed guidance is provided. If all PCR and Immunofluorescence tests are negative and no virus is subtypable, samples are to be sent to the Atlanta Centers for Disease Control for further unspecified testing. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

53. **Egypt:** The data obtained included information from the Ministry of Health and Population,[528] a published article on the national disease surveillance system which details the number of diseases under surveillance,[529] and a published article on the national pandemic preparedness plan for acute respiratory infections.[530] There is no relevant public health legislation available. No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. Egypt completed a WHO Joint External Evaluation in November 2018, but there has not been a published report as of 31 December 2022.[300]

54. **El Salvador:** The data obtained included information from the Ministry of Health,[531] the latest epidemiological bulletin,[532] the *Health Code,*[533] and the National Reference Laboratory.[534] The epidemiological surveillance system guidelines contains the nationally notifiable disease list and specifies reporting of emerging diseases, as well as unusual respiratory infections with unknown pathogens.[535] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

55. **Equatorial Guinea:** The data obtained included information from the Ministry of Health and Social Welfare,[536] and health system strengthening guidance.[537] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. There is no relevant public health legislation available. Equatorial Guinea completed a WHO Joint External Evaluation in December 2019, but there has not been a published report as of 31 December 2022.[300]

56. **Eritrea:** The data obtained included information from the country's published WHO Joint External Evaluation report,[538] and the Ministry of Information.[539] There is no Ministry of Health, Public Health, or national laboratory presence online, as was also found by the 2021 GHSI.[48] There is no relevant public health legislation available. No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[538]

57. **Estonia:** The data obtained included information from the national Health Board,[540] the *Prevention and Control of Infectious Diseases Act* which considers new dangerous infectious diseases,[541] the latest published disease surveillance data,[542] and the nationally notifiable disease list in Ministry of Health Regulations which includes

reporting requirements for new diseases.[543] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

58. **Eswatini:** The data obtained included information from the Ministry of Health,[544] the country's published WHO Joint External Evaluation report,[545] and the *Public Health Act*.[546] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[545]

59. **Ethiopia:** The data obtained included information from the Ministry of Health,[547] the country's published WHO Joint External Evaluation report,[548] the nationally notifiable disease list,[549] and the latest epidemiological bulletin.[550] The community and event-based surveillance manual mentions the need to report unusual and unknown diseases including unexplained deaths.[551]

60. **Fiji:** The data obtained included information from the Ministry of Health,[552] the national Communicable Disease Surveillance and Outbreak Response Guidelines including nationally notifiable disease list,[553] and the *Public Health Act*.[554] There is health professional guidance provided on emerging infectious diseases and novel pathogens.[553] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

61. **Finland:** The data obtained included information from the Ministry of Social Affairs and Health,[555] the country's published WHO Joint External Evaluation report,[556] the nationally notifiable disease list,[557] published infectious disease surveillance data,[558] and the *Infectious Diseases Act*.[559]

62. **France:** The data obtained included information from the Ministry of Health,[560] the notifiable disease surveillance system,[561] and the list of notifiable diseases.[562] France releases weekly epidemiological bulletins which contain public health publications but not nationally notifiable disease surveillance data routinely.[563] Disease surveillance data is published for notifiable diseases via an online platform run by Public Health France.[564] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

63. **Gabon:** The data obtained included information from the Ministry of Health and Social Affairs,[565] the country's published WHO Joint External Evaluation report,[566] and the national health development plan.[567] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[566] There is no relevant public health legislation available, and this was confirmed also in the WHO Joint External Evaluation report.

64. **Gambia:** The data obtained included information from the Ministry of Health,[568] the *Public Health Act*,[569] and the country's published WHO Joint External Evaluation report.[570] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[566]

65. **Georgia:** The data obtained included information from the Ministry of Health,[571] the country's published WHO Joint External Evaluation report,[572] the Electronic Integrated Disease Surveillance System,[573] the *Public Health Law* with 2022 amendments,[574] national Global health security agenda pilot assessment,[575] and the nationally notifiable disease list with latest disease surveillance data.[576]

66. **Germany:** The data obtained included information from the Federal Ministry of Health,[577] the national pandemic plan,[578] the latest epidemiological bulletin,[579] a webpage on the International Health Regulations mentioning reporting requirements for diseases with unknown causes,[580] and the *Infection Protection Act*.[581] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

67. **Ghana:** The data obtained included information from the Ministry of Health,[582] the country's published WHO Joint External Evaluation report,[583] national laboratory guidelines,[584] communicable disease guidelines,[585] latest epidemiological bulletin containing the nationally notifiable disease list,[586] and the national *Public Health Act*.[587] The nationally notifiable disease list includes "public health event of international concern" but does not provide further guidance or detail new or unknown diseases.[586]

68. **Greece:** The data obtained included information from the Ministry Of Health And Social Solidarity,[588] the *Reformulation of the list of mandatory notifiable diseases*,[589] and the latest published disease surveillance data.[590] The national communicable disease action plan mentions disease outbreaks of unknown origin but does not provide further guidance.[591] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

69. **Grenada:** The data obtained included information from the Ministry Of Health,[592] the *Public Health Act* including list of nationally notifiable diseases,[593] and a publication on notifiable disease reporting in Grenada.[594] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

70. **Guatemala:** The data obtained included information from the Ministry of Public Health and Social Assistance,[595] epidemiological monitoring protocol with nationally notifiable disease list,[596] the national disease surveillance system manual,[597] the *Health Code,*[598] and latest epidemiological bulletin.[599] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

71. **Guinea:** The data obtained included information from the Ministry of Health,[600] the country's published WHO Joint External Evaluation report,[601] list of nationally notifiable diseases,[602] the *Public Health Code*,[603] and the latest epidemiological bulletin.[604]

72. **Guinea-Bissau:** The data obtained included information from the country's published WHO Joint External Evaluation report,[605] the *Anti-economic and public health offences* decree,[606] and a published article on the national health development plan.[607] The Ministry of Public Health's online presence is limited to an official Facebook page.[608] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible.

73. **Guyana:** The data obtained included information from the Ministry of Health,[609] the *Public Health Act*,[610] and the COVID-19 emergency response plan.[611] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

74. **Haiti:** The data obtained included information from the Ministry of Public Health and Population,[612] the latest epidemiological report which contains the nationally notifiable disease list,[613] laboratory guidelines,[614] the *Decree on the organisation and functioning of the Ministry of Public Health and Population*,[615] and a published article on the national disease surveillance system.[616] The nationally notifiable disease list includes reporting requirements for "unusual phenomenon" but does not specify new or unknown diseases.[613] A WHO Joint External Evaluation was completed in July 2019, but no report has been made available as of 31 December 2022.[300]

75. **Honduras:** The data obtained included information from the Ministry of Health,[617] the *Health Code*,[618] and the influenza pandemic plan.[619] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

76. **Hungary:** The data obtained included information from the Ministry of Health,[620] the *Decree on the necessary epidemiological measures to prevent infectious diseases and epidemics* which includes diseases of unknown aetiology,[621] the latest weekly epidemiological bulletin,[622] and the reporting requirements for infectious diseases with nationally notifiable disease list.[623]

77. **Iceland:** The data obtained included information from the Ministry of Health,[624] Directorate of Health,[625] *Epidemic Prevention Act* which includes events of unknown cause or origin,[626] the nationally notifiable disease list,[627] the latest published disease

data report,[628] and disease notification instructions for doctors.[629] Reporting guidelines for doctors and laboratories include unusual and unexpected events.[630] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

78. **India:** The data obtained included information from the Ministry of Health and Family Welfare,[631] list and reporting form for notifiable diseases,[632] the *Epidemic Diseases Act*,[633] the latest published epidemiology report,[634] and reporting forms for syndromic surveillance,[635] and for unusual cases, deaths and events.[156] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

79. **Indonesia:** The data obtained included information from the Ministry of Health,[636] the country's published WHO Joint External Evaluation report,[637] the latest published national disease surveillance data,[638] the decree on the *National Health System*,[639] and *Regulations* for the Ministry of Health containing the nationally notifiable disease list.[640] Health professional guidelines include information and instructions on reporting unknown diseases.[641]

80. **Iran:** The data obtained included information from the Ministry of Health and Medical Education,[642] the *Law on the prevention of communicable diseases* which includes the nationally notifiable disease list,[643] and published articles on the national infectious disease surveillance system.[644,645] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

81. **Iraq:** The data obtained included information from the Ministry of Health,[646] the country's published WHO Joint External Evaluation report,[647] the *Public Health Law*,[648] and the latest epidemiological bulletin.[649] A published article on the communicable disease surveillance system specifies the number of nationally notifiable diseases but no list is publicly available.[650]

82. **Ireland:** The data obtained included information from the Department of Health,[651] the nationally notifiable disease list,[652] the *Infectious Diseases (Amendment) Regulations*,[653] infectious disease outbreak reporting form which includes unknown disease outbreaks,[654] and the latest published disease outbreak surveillance data.[655] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

83. **Israel:** The data obtained included information from the Ministry of Health,[656] the *Public Health Ordinance* which includes unknown diseases,[657] the *Public Health Regulations*,[658] the latest published national disease surveillance data,[659] and notifiable infectious diseases publication with nationally notifiable disease list which includes diseases of unknown cause.[660] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

84. **Italy:** The data obtained included information from the Ministry of Health,[661] the pandemic influenza plan,[662] the *Ministry of Health Decree*,[663] national disease notification instructions,[664] and the nationally notifiable disease list.[665] National surveillance data is released variably for different diseases and disease groups.[666] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

85. **Jamaica:** The data obtained included information from the Ministry of Health and Wellness,[667] the latest published national disease surveillance data which contains the nationally notifiable disease list,[668] and the *Public Health Act*.[669] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

86. **Japan:** The data obtained included information from the Ministry of Health, Labour and Welfare,[670] the country's published WHO Joint External Evaluation report,[671] National Institute for Infectious Diseases laboratory manuals,[672] *Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases*,[673] and the latest published national disease surveillance data.[674] The infectious disease surveillance system guidelines include unknown pathogens.[675]

87. **Jordan:** The data obtained included information from the Ministry of Health,[676] the country's published WHO Joint External Evaluation report,[677] the *Public Health Law*,[678] and overview of the national public health surveillance system.[679] No complete nationally notifiable disease list was publicly accessible, but a published article specifies the number of diseases under surveillance.[680] No nationally published disease surveillance data were available.

88. **Kazakhstan:** The data obtained included information from the Ministry of Healthcare,[681] the legal code *On the health of the people and the healthcare system*,[682] the latest published national disease surveillance data,[683] and a published article which specifies the number of diseases under national surveillance but no complete list is available.[684] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

89. **Kenya:** The data obtained included information from the Ministry of Health,[685] the country's published WHO Joint External Evaluation report,[686] the *Public Health Act*,[687] and national surveillance guidelines.[688] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[686]

90. **Kiribati:** The data obtained included information from the Ministry of Health and Medical Services,[689] a national Communicable Disease Surveillance Newsletter,[690] and the *Public Health (Infectious Disease) Regulations*.[691] No complete nationally

notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

91. **Kuwait:** The data obtained included information from the Ministry of Health,[692] and the country's published WHO Joint External Evaluation report.[693] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. There is no relevant public health legislation available, and this was also confirmed in the WHO Joint External Evaluation report.[693]

92. **Kyrgyz Republic:** The data obtained included information from the Ministry of Health,[694] the country's published WHO Joint External Evaluation report,[695] the resolution *On the Concept of Comprehensive Protection of the Population,*[696] the decree *On approval of the Agreement on Cooperation between the Member States of the Eurasian Economic Community in the field of combating infectious diseases*,[697] and the latest published national disease surveillance data which contains the nationally notifiable disease list.[698]

93. **Laos:** The data obtained included information from the Ministry of Health,[699] the country's published WHO Joint External Evaluation report,[700] the *Law On Hygiene, Disease Prevention And Health Promotion*,[701] the most recent national Health Strategy.[702] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

94. **Latvia:** The data obtained included information from the Ministry of Health,[703] the country's published WHO Joint External Evaluation report,[704] the *Epidemiological Safety Law*,[705] the *Procedure for registration of infectious diseases* which contains the nationally notifiable disease list,[706] disease reporting guidelines,[707] and the latest published national disease surveillance data.[708] Guidelines on reporting outbreak clusters include "severe acute infectious diseases of uncertain aetiology with five or more patients hospitalised" mandatory notification requirements.[154]

95. **Lebanon:** The data obtained included information from the Ministry of Public Health,[709] the country's published WHO Joint External Evaluation report,[710] Notifiable Communicable Diseases Surveillance Guidelines which contains the nationally notifiable disease list,[711] the latest published national disease surveillance data,[712] and the *Infectious Diseases Law*.[713]

96. **Lesotho:** The data obtained included information from the Ministry of Health,[714] the country's published WHO Joint External Evaluation report,[715] and the *Public Health Order* which contains the nationally notifiable disease list. [716] No nationally published disease surveillance data were available.

97. **Liberia:** The data obtained included information from the Ministry of Health,[717] the National Health Public Institute Of Liberia,[718] the latest published national disease surveillance data,[719] and the *Public Health Law* which contains the nationally notifiable disease list and includes unexplained and unknown clusters of health events and deaths.[151]

98. **Libya:** The data obtained included information from the Ministry of Health,[720] the country's published WHO Joint External Evaluation report,[721] the *Health Law*,[722] and the latest epidemiological bulletin.[723] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[721]

99. **Liechtenstein:** The data obtained included information from the Federal Office of Public Health,[724] the country's published WHO Joint External Evaluation report,[725] the Influenza Pandemic Plan,[726] the latest published national disease surveillance data,[727] and the *Ordinance on the reporting of observations of communicable diseases in humans* which contains the nationally notifiable disease list and reporting requirements for unusual and unexpected infectious diseases.[728] Reporting guidelines for health professionals and laboratories include new, unusual or unexpected health threats and new, unknown pathogens.[729] Liechtenstein collaborates with Switzerland for public health functions and shares infectious disease reporting structures and guidelines.[725]

100. **Lithuania:** The data obtained included information from the Ministry of Health Protection,[730] the country's published WHO Joint External Evaluation report,[731] the latest published national disease surveillance data,[732] the *Law on the Prevention and Control of Communicable Diseases in Humans* which includes communicable diseases of unknown origin,[733] and the nationally notifiable disease list.[734]

101. **Luxembourg:** The data obtained included information from the Ministry of Health,[735] the National Health Laboratory,[736] the mandatory disease reporting guidelines,[737] the *Law of 1 August 2018 on the compulsory notification of certain diseases in the context of the protection of public health*,[738] and the *Regulation of 15 February 2019 listing the diseases subject to compulsory notification*.[739] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

102. **Madagascar:** The data obtained included information from the country's published WHO Joint External Evaluation report,[740] and the *Health Code*.[741] The Ministry of Public Health and Ministry of Justice websites were inaccessible at time of research.[742,743] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

103. **Malawi:** The data obtained included information from the Ministry of Health,[744] the country's published WHO Joint External Evaluation report,[745] and the *Public Health Act* which contains the nationally notifiable disease list.[746] No nationally published disease surveillance data were available.

104. **Malaysia:** The data obtained included information from the Ministry of Health,[747] the country's published WHO Joint External Evaluation report,[748] and the latest published national disease surveillance data.[749] The national surveillance guidelines which includes the nationally notifiable disease list,[750] and the *Prevention And Control Of Infectious Diseases Act*[751] include "any other" life threatening infectious disease, which has subsequently been interpreted by the Malaysian Government to include new diseases and applied to novel pathogens such as the emergence of SARS-CoV-2 during the COVID-19 pandemic.[752] The national laboratory guidelines explicitly outlines an approach to "unknown virus infection" with algorithms and protocols.[753] The Standard Operating Procedures for Potential Infectious Disease includes unexplained deaths and "emerging or unknown infections" but does not provide further detail.[754]

105. **Maldives:** The data obtained included information from the Ministry of Health,[755] the country's published WHO Joint External Evaluation report,[756] the *Public Health Protection Act*,[757] and national health guidelines.[758] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

106. **Mali:** The data obtained included information from the country's published WHO Joint External Evaluation report,[759] and the decree *Operation Of The Institute National Health Research Public*.[760] The Ministry of Health and Public Hygiene website was inaccessible at time of research.[761] No complete nationally notifiable disease list was publicly accessible.No nationally published disease surveillance data were available.

107. **Malta:** The data obtained included information from the Ministry of Health,[762] communicable disease guidelines,[763] the nationally notifiable disease list,[764] the *Public Health Act*,[765] and latest published national disease surveillance data.[766] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

108. **Marshall Islands:** The data obtained included information from the Ministry of Health and Human Services,[767] the country's published WHO Joint External Evaluation report,[768] and the *Public Health, Safety and Welfare Act*.[767] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[768]

109. **Mauritania:** The data obtained included information from the Ministry of Health,[769] the country's published WHO Joint External Evaluation report,[770] the latest published national disease surveillance data,[771] and the decree S*etting the powers of the Minister of Health and the organisation of the central administration of his department*.[772] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

110. **Mauritius:** The data obtained included information from the Ministry of Health and Wellness,[773] the country's published WHO Joint External Evaluation report,[774] the *Public Health Act*,[775] and the latest published national disease surveillance data.[776] No complete nationally notifiable disease list was publicly accessible.

111. **Mexico:** The data obtained included information from the Ministry of Health,[777] and the latest published national disease surveillance data.[778] The *Standard for Epidemiological Surveillance*, which contains the nationally notifiable disease list, also includes new emerging diseases and events.[779] The Disaster Health Care Manual briefly mentions outbreaks caused by previously unknown diseases.[780] Laboratory guidance for novel pathogens exists in the national influenza pandemic plan but only for influenza.[781] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

112. **Micronesia, Federated States of:** The data obtained included information from the Department of Health and Social Affairs,[782] Government Of The Federated States Of Micronesia,[783] the country's published WHO Joint External Evaluation report,[784] *Title 41 Public Health, Safety and Welfare*,[785] and the national COVID-19 Response Framework.[786] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

113. **Moldova:** The data obtained included information from the Ministry of Health,[787] the country's published WHO Joint External Evaluation report,[788] the latest published national disease surveillance data,[789] and the law *Regarding State Supervision Of Public Health*.[790] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[788]

114. **Monaco:** The data obtained included information from the Ministry of Health and Social Affairs,[791] and public health system.[792] The *Ordinance no. 3.153* includes unexpected or unusual events regardless of source.[793] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

115. **Mongolia:** The data obtained included information from the Ministry of Health,[794] the country's published WHO Joint External Evaluation report,[795] the National Centre for Communicable Disease,[796] the *Law on Health*, and the latest published national disease surveillance data.[797] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[795]

116. **Montenegro:** The data obtained included information from the Ministry of Health,[798] Institute for Public Health,[799] the country's published WHO Joint External Evaluation report,[800] the Health Statistical Yearbook,[801] and the latest published national disease surveillance data.[802] The law on the *Protection of the Population from Infectious Diseases* which contains the nationally notifiable disease list includes unknown diseases and outbreaks of unknown aetiology, but not on the list itself.[803]

117. **Morocco:** The data obtained included information from the Ministry of Health and Social Protection,[804] the country's published WHO Joint External Evaluation report,[805] the latest published national disease surveillance data,[806] and the Institut Pasteur Morocco.[807] The *Order of the Minister of Public Health No. 683-95,* which contains the nationally notifiable disease list, includes illnesses of unknown cause, but not on the list itself.[808]

118. **Mozambique:** The data obtained included information from the Ministry of Health,[809] the country's published WHO Joint External Evaluation report,[810] the Strategic Plan for the Health Sector,[811] and the latest published national disease surveillance data which contains the nationally notifiable disease list.[812]

119. **Myanmar:** The data obtained included information from the Ministry of Health,[813] the country's published WHO Joint External Evaluation report,[814] the national action plan for health security,[815] the law on *Prevention and Control of Communicable Diseases*,[816] and its 2011 amendment,[817] the latest published national disease surveillance data which contains the nationally notifiable disease list.[818]

120. **Namibia:** The data obtained included information from the Ministry of Health,[819] the country's published WHO Joint External Evaluation report,[820] and the *Public and Environmental Health Act* which contains the nationally notifiable disease list.[821] No nationally published disease surveillance data were available.

121. **Nauru:** The data obtained included information from the Government of the Republic of Nauru,[822] the Health Sector Strategic Plan 2010–2015,[823] and the *Notification of Infectious and Contagious Diseases Ordinance* which mandates all infectious diseases must be reported without a list provided.[824] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

122. **Nepal:** The data obtained included information from the Ministry of Health and Population,[825] the Surveillance Of Communicable Disease Program,[826] the latest published national disease surveillance data,[827] the *Infectious Diseases Act*,[828] A surveillance system review publication states the number of diseases under national surveillance but a complete list is not publicly accessible.[829]

123. **Netherlands:** The data obtained included information from the Ministry of Health, Welfare and Sport,[830] the nationally notifiable disease list,[831] and the latest published national disease surveillance data.[832] The Scenario management of infectious disease playbook includes unknown infectious diseases.[833] The *Public Health Act* includes new and unknown diseases.[834] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

124. **New Zealand:** The data obtained included information from the Ministry of Health,[835] the country's published WHO Joint External Evaluation report,[836] list of nationally notifiable diseases,[837] the *Health Act 1956*,[838] and the latest published national disease surveillance data.[839] The Guidelines for the Investigation and Control of Disease Outbreaks includes guidance for health professionals on diseases of unknown cause and provides a form. [840] The role of the laboratory is briefly mentioned but no detailed laboratory guidance is provided.

125. **Nicaragua:** The data obtained included information from the Ministry of Health,[841] the latest published national disease surveillance data,[842] and *General Health Law.*[843] No complete nationally notifiable disease list was publicly accessible. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

126. **Niger:** The data obtained included information from the Ministry of Public Health, Population and Social Affairs,[844] the country's published WHO Joint External Evaluation report,[845] and an overview of the Ministry of Public Health.[846] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[845] There is no relevant public health legislation available, and this was also confirmed in the WHO Joint External Evaluation report.

127. **Nigeria:** The data obtained included information from the Federal Ministry of Health,[847] the country's published WHO Joint External Evaluation report,[848] the Nigeria Centre for Disease Control,[849] the latest published national disease surveillance data,[850] a published article on the disease surveillance system,[851] and the *National Health Act 2014*.[852] The national surveillance guidelines, which contains the nationally notifiable disease list, includes new and unknown infectious disease threat guidance and a form, but unknown diseases are not on the list itself.[853]

128. **Niue:** The data obtained included information from the Government of Niue,[854] the *Public Health Act* which includes a list of nationally notifiable diseases,[855] and the Health Strategic Plan 2011-2021.[856] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

129. **North Korea:** The data obtained included information from a published systematic review on the national public health system,[857] and the *People's Public Health Law of the Democratic People's Republic of Korea 2012*.[858] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

130. **North Macedonia:** The data obtained included information from the Ministry of Health,[859] the country's published WHO Joint External Evaluation report,[860] and the latest published national disease surveillance data.[861] The *Law On The Protection Of The Population Against Infectious Diseases* which contains the nationally notifiable disease list, has listed "unknown diseases" and mentions new unknown diseases outside of the list as well.[862]

131. **Norway:** The data obtained included information from the Ministry of Health and Care Services,[863] the National Health Preparedness Plan,[864] the *Act on Protection against Infectious Diseases*,[865] the nationally notifiable disease list,[866] and the latest published national disease surveillance data.[867] Guidance is provided on "infectious disease that may be of importance to international public health" but new diseases and unknown pathogens are not specified.[868]

132. **Oman:** The data obtained included information from the Ministry of Health,[869] the country's published WHO Joint External Evaluation report,[870] the latest published national disease surveillance data which contains the nationally notifiable disease list,[871] and *The Law on the Control of Communicable Diseases*.[872] The Manual on Communicable Diseases includes reporting requirements for diseases of unknown aetiology, and defines clusters as "3 cases or more of a disease of unknown aetiology in a health care facility, village, community, construction site, mine, school or other institution over a period of 2 weeks" and provides reporting guidance.[153]

133. **Pakistan:** The data obtained included information from the Ministry of National Health Services Regulations & Coordination,[873] the National Institutes of Health,[874] the country's published WHO Joint External Evaluation report,[875] the nationally notifiable disease list,[876] the *Epidemic Diseases Act*,[877] and the latest published national disease surveillance data.[878]

134. **Palau:** The data obtained included information from the Ministry of Health and Human Services,[879] the country's published WHO Joint External Evaluation report,[880] the National Health Profile,[881] and the latest published national disease surveillance data.[882] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[880] There is no relevant public health legislation available, and this was also confirmed in the WHO Joint External Evaluation report.

135. **Panama:** The data obtained included information from the Ministry of Health,[883] the latest published national disease surveillance data,[884] The *Executive Decree That Determines Mandatory Notification Health Problems, Points Out The Procedures for Notification and Establish Sanctions* contains the nationally notifiable disease list and includes emerging diseases.[885]

136. **Papua New Guinea:** The data obtained included information from the Ministry of Health,[886] and the *Public Health Act* which contains the nationally notifiable disease list.[887] The latest National Health Plan mentions emerging infections but does not provide further guidance.[888] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

137. **Paraguay:** The data obtained included information from the Ministry of Health and Social Welfare,[889] the latest published national disease surveillance data,[890] the National surveillance Guide which contains the nationally notifiable disease list,[891] and the *Sanitary Code*.[892] The national surveillance guide includes guidance on "emerging or unknown diseases" and provides a specific form for reporting and investigating.[891] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

138. **Peru:** The data obtained included information from the Ministry of Health,[893] the National Institute of Health,[894] outbreak notification guidelines,[895] tools for epidemiological surveillance,[896] the nationally notifiable disease list,[897] the *General Health Law*,[898] and the latest published national disease surveillance data.[899]

139. **Philippines:** The data obtained included information from the Department of Health,[900] the country's published WHO Joint External Evaluation report,[901] the *Mandatory Reporting of Notifiable Diseases and Health Events of Public Health Concern Act* which contains the nationally notifiable disease list,[902] and the latest published national disease surveillance data.[903] Legislation includes diseases "caused by previously undetected or unknown infectious agents" but not in the nationally notifiable list itself.[902]

140. **Poland:** The data obtained included information from the Ministry of Health,[904] Department of Epidemiology of Infectious Diseases and Surveillance,[905] the latest published national disease surveillance data,[906] and the Act *On preventing and combating infections and infectious diseases in humans* which contains the nationally notifiable disease list.[907]

141. **Portugal:** The data obtained included information from the Directorate-General for Health,[908] the Public Health Emergency Center,[909] the nationally notifiable disease list,[910] the latest published national disease surveillance data,[911] the *Law nº 81/2009 of 21 August*,[912] and the *Order No. 15385-A/2016 of December 21st* which contains the nationally notifiable disease list.[913] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

142. **Qatar:** The data obtained included information from the Ministry of Public Health,[914] the country's published WHO Joint External Evaluation report,[915] the Decree *On Protection from Infectious Diseases* which contains the nationally notifiable disease list,[916] and the latest published national disease surveillance data.[917]

143. **Romania:** The data obtained included information from the Ministry of Health,[918] the National Institute Of Public Health,[919] the National Center for Surveillance and Control of Communicable Diseases,[920] and the latest published national disease surveillance data.[921] The decision No 589 *Regarding the establishment of the reporting and data collection methodology for the surveillance of communicable diseases* contains the nationally notifiable disease list and diseases of "unknown aetiology" are included.[922] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

144. **Russia:** The data obtained included information from the Ministry of Health,[923] the latest published national disease surveillance data,[924] and the *On Approval Of Forms Of Federal Statistical Observation With Instructions For Completing Them For The Federal Service For Supervision Of Consumer Rights Protection And Human Welfare To Organise Federal Statistical Observation Of The Sanitary Condition Of A Subject Of The Russian Federation* order which contains the nationally notifiable disease list.[925] The regulations *Prevention of Infectious Diseases* includes health professional guidelines and mentions diseases of unknown aetiology.[150] The *On The Optimization Of Anti-epidemic Work And The Approval Of The Form Of The Act Of The Epidemiological Investigation Of The Focus Of An Infectious (Parasitic) Disease With The Establishment Of A Causal Relationship* briefly mentions the role of the laboratory, including updating laboratories with modern equipment for enzyme immunoassay and molecular genetic analysis, but does not provide further details for new diseases.[926]

145. **Rwanda:** The data obtained included information from the Ministry of Health,[927] the country's published WHO Joint External Evaluation report,[928] the latest Health Sector Strategic Plan,[929] and the latest published national disease surveillance data which contains the nationally notifiable disease list.[930] There is no relevant public health legislation available.[931]

146. **Samoa:** The data obtained included information from the Ministry of Health,[932] a Pacific Health Security scoping mission report,[933] the *Ministry of Health Act*,[934] a published article on national surveillance,[935] and the latest published national disease surveillance data.[936] The National Pandemic Plan briefly mentions emerging disease threats.[937] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

147. **San Marino:** The data obtained included information from the Secretariat of State for Health and Social Security,[938] the Social Security Institute,[939] the *Ordinances of the Secretary of State for Health and Social Security*,[940] the nationally notifiable disease list,[941] and the latest published national disease surveillance data.[942] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

148. **São Tomé and Príncipe:** The data obtained included information from the Ministry of Health,[943] the country's published WHO Joint External Evaluation report,[944] the latest published national disease surveillance data,[945] the *Basic Health Law*,[946] and the Procedures Manual for the National Health Service.[947] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation states there is one that exists internally.[944]

149. **Saudi Arabia:** The data obtained included information from the Ministry of Health,[948] the country's published WHO Joint External Evaluation report,[949] the latest published national disease surveillance data,[950] and the *Health System* Royal Decree.[951] The public health surveillance technical guidelines contains the nationally notifiable disease list, which requires reporting of any emerging disease.[952]

150. **Senegal:** The data obtained included information from the Ministry of Health and Social Action,[953] the country's published WHO Joint External Evaluation report,[954] the latest published national disease surveillance data,[955] and the *Decree on the organization of the Ministry of Health and Social Action*.[956] The national guide for integrated monitoring disease and response which contains the nationally notifiable disease list includes emerging diseases, clusters of unknown disease outbreaks and unexplained deaths and provides guidance and a form.[152]

151. **Serbia:** The data obtained included information from the Ministry of Health,[957] the country's published WHO Joint External Evaluation report,[958] and the latest published national disease surveillance data.[959] The *Law on the Protection of the Population from Infectious Diseases* contains the nationally notifiable disease list and

includes emerging infectious diseases and unknown causative agents and briefly mentions the role of the laboratory. [960] There is a rulebook on reporting infectious diseases which includes unknown diseases,[961] and there is a specific reporting form.[962]

152. **Seychelles:** The data obtained included information from the Ministry of Health,[963] the country's published WHO Joint External Evaluation report,[964] the *Public Health Act*,[965] the National Health Strategic Plan 2022-2026 which contains the nationally notifiable disease list,[966] and the latest published national disease surveillance data.[967]

153. **Sierra Leone:** The data obtained included information from the Ministry of Health and Sanitation,[968] the country's published WHO Joint External Evaluation report,[969] the *Public Health Ordinance* which contains the nationally notifiable disease list,[970] *The Public Health (Amendment) Act*,[971] the latest National Health Sector Strategic Plan,[972] and the latest published national disease surveillance data.[973] The National Community Health Worker Policy briefly mentions reporting requirements for any unusual events and clustered deaths.[974]

154. **Singapore:** The data obtained included information from the Ministry of Health,[975] the country's published WHO Joint External Evaluation report,[976] the latest published national disease surveillance data,[977] the nationally notifiable disease list,[978] and the *Infectious Disease Act*.[979] The Pandemic readiness and response plan considers laboratory testing for novel respiratory pathogens but does not provide a protocol or cover other unknown diseases.[980] Singapore requires notification of "clusters of infectious diseases, suspected bioterrorism agents, suspect cases of emerging infectious diseases" but does not include these directly in the nationally notifiable disease list.[978] As detailed in the national Communicable Diseases Surveillance guidelines, Singapore operates a Severe Illness and Death from Possibly Infectious Causes programme to detect unexplained deaths and identify novel, emerging diseases, providing health professional guidance.[981]

155. **Slovakia:** The data obtained included information from the Ministry of Health,[982] and the latest published national disease surveillance data.[983] The *Act on the Protection, Support and Development of Public Health* which contains the nationally notifiable disease list and includes unknown diseases.[984] The pandemic plan includes guidance for new viruses for health professionals and laboratories but only provides a laboratory protocol for COVID-19 and influenza, not novel pathogens.[985] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

156. **Slovenia:** The data obtained included information from the Ministry of Health,[986] the country's published WHO Joint External Evaluation report,[987] the nationally notifiable disease list,[988] the latest published national disease surveillance data through the data portal,[989] the Epidemiological monitoring of infectious diseases in Slovenia in 2019 and 2020 report,[990] the latest annual health statistical yearbook,[991] and the

Infectious Diseases Act.[992] The national plan for epidemics and pandemics provides guidelines on new diseases for health professionals and laboratories.[990]

157. **Solomon Islands:** The data obtained included information from the Ministry of Health and Medical Services,[993] a published article on the syndromic surveillance system,[994] the latest National Health Strategic Plan,[995] a Health Security Scoping Mission report detailing the syndromic surveillance system,[996] and the latest Statistical Health Core Indicator Report from 2018.[997] No complete nationally notifiable disease list was publicly accessible. There is no relevant public health legislation available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

158. **Somalia:** The data obtained included information from the Ministry of Health,[998] the country's published WHO Joint External Evaluation report,[999] a published article which contains the nationally notifiable disease list,[1000] and the latest national health sector strategic plan,[1001] No nationally published disease surveillance data were available. There is no relevant public health legislation available, and this was confirmed also in the WHO Joint External Evaluation report.[999]

159. **South Africa:** The data obtained included information from the National Department of Health,[1002] the country's published WHO Joint External Evaluation report,[1003] and the latest published national disease surveillance data.[1004] The *National Health Act,* which contains the nationally notifiable disease list, requires reporting of unusual cases and disease clusters and novel respiratory pathogens.[1005] Guidelines provide detailed health professional guidance for reporting novel respiratory pathogens and previously unrecognised pathogens and mention the role of the laboratory.[1006]

160. **South Korea:** The data obtained included information from the Ministry of Health and Welfare,[1007] the country's published WHO Joint External Evaluation report,[1008] the latest published national disease surveillance data,[1009] and the nationally notifiable disease list.[1010] The *Infectious Disease Control and Prevention Act* includes coverage of new pathogens.[1011] The latest Infectious Disease Prevention and Management Basic Plan includes guidelines for health professionals and laboratories on new and unknown diseases.[1012]

161. **South Sudan:** The data obtained included information from the Ministry of Health,[1013] the country's published WHO Joint External Evaluation report,[1014] and the latest National Health Policy.[1015] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. There is no relevant public health legislation available, and this was confirmed also in the WHO Joint External Evaluation report.[1014]

162. **Spain:** The data obtained included information from the Ministry of Health,[1016] the National Early Warning and Rapid Response System,[1017] the *Order which creates the National Epidemiological Surveillance Network, relating to the list of notifiable diseases, methods of notification and regional endemic diseases* which contains the nationally notifiable disease list,[1018] and the latest published national disease surveillance data.[1019] The Carlos III Institute mentions reporting requirements for outbreaks of any aetiology.[1020]

163. **Sri Lanka:** The data obtained included information from the Ministry of Health,[1021] the country's published WHO Joint External Evaluation report,[1022] the latest published national disease surveillance data,[1023] the *Quarantine and Prevention of Diseases*,[1024] and the nationally notifiable disease list.[1025]

164. **St Kitts & Nevis:** The data obtained included information from the Ministry of Health,[1026] the *Public Health Act*,[1027] and the *Public Health (Quarantine) Regulations* which contains the nationally notifiable disease list.[1028] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

165. **St Lucia:** The data obtained included information from the Ministry of Health, Wellness and Elderly Affairs,[1029] the *Public Health Act*,[1030] and the *Public Health (Communicable and Notifiable Diseases)* regulations which contains the nationally notifiable disease list.[1031] The National Communicable Disease Surveillance Manual includes guidelines for health professionals on outbreaks with unknown aetiology, providing guidance, a flow chart and forms, and mentions the role of the laboratory.[1032] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

166. **St Vincent & The Grenadines:** The data obtained included information from the Ministry of Health, Wellness and the Environment,[1033] a Health Systems assessment,[1034] and the *Public Health (Public Bodies Special Measures) Rules*.[1035] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

167. **Sudan:** The data obtained included information from the Federal Ministry of Health,[1036] the country's published WHO Joint External Evaluation report,[1037] the *National Public Health Act*,[1038] the nationally notifiable disease list,[1039] and the latest published national disease surveillance data.

168. **Suriname:** The data obtained included information from the Ministry of Health,[1040] the National Pandemic Preparedness Plan,[1041] and the *Law Of Public Health*.[1042] No complete nationally notifiable disease list was publicly accessible. No

nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

169. **Sweden:** The data obtained included information from the Ministry of Health and Social Affairs,[1043] the Public Health Agency,[1044] the nationally notifiable disease list,[1045] the latest published national disease surveillance data,[1046] and the *Infection Prevention Act*.[1047]

170. **Switzerland:** The data obtained included information from the Federal Office of Public Health,[724] the country's published WHO Joint External Evaluation report,[725] the Influenza Pandemic Plan,[726] the latest published national disease surveillance data,[727] and the *Ordinance on the reporting of observations of communicable diseases in humans* which contains the nationally notifiable disease list and reporting requirements for unusual and unexpected infectious diseases.[728] Reporting guidelines for health professionals and laboratories include new, unusual or unexpected health threats and new, unknown pathogens.[729] Switzerland collaborates with Liechtenstein for public health functions and shares infectious disease reporting structures and guidelines.[725]

171. **Syria:** The data obtained included information from the Ministry of Health,[1048] the latest published national disease surveillance data,[1049] and latest WHO national epidemiological bulletin.[1050] No complete nationally notifiable disease list was publicly accessible. There is no relevant public health legislation available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

172. **Tajikistan:** The data obtained included information from the Ministry of Health and Social Protection of Population,[1051] the country's published WHO Joint External Evaluation report,[1052] the *Health Code*,[1053] and the latest published national disease surveillance data.[1054] No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[1052]

173. **Tanzania:** The data obtained included information from the Ministry of Health,[1055] the country's published WHO Joint External Evaluation report,[1056] the published WHO Joint External Evaluation report for Zanzibar,[1057] and the *Public Health Act* which contains the nationally notifiable disease list.[1058] No nationally published disease surveillance data were available.

174. **Thailand:** The data obtained included information from the Ministry of Public Health,[1059] the country's published WHO Joint External Evaluation report,[1060] the latest published national disease surveillance data,[1061] and the *Communicable Diseases Act*,[1062] and reporting guidelines which contains the nationally notifiable disease list.[1063] There are health professional guidelines,[1064] and laboratory guidelines,[1065] for

emerging infectious diseases, although these mostly focus on known pathogens and only briefly cover guidance on new diseases.

175. **Timor-Leste:** The data obtained included information from the Ministry of Health,[1066] the country's published WHO Joint External Evaluation report,[1067] and the latest National Health Sector Plan.[1068] The Decree law *System of Epidemiological Surveillance* includes surveillance requirements for new diseases.[1069] No nationally published disease surveillance data were available. No complete nationally notifiable disease list was publicly accessible, but the WHO Joint External Evaluation report specifies the number of diseases under surveillance.[1067]

176. **Togo:** The data obtained included information from the Ministry Of Health, Public Hygiene And Universal Access To Healthcare,[1070] the country's published WHO Joint External Evaluation report,[1071] and the *Public Health Code*.[1072] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

177. **Tonga:** The data obtained included information from the Ministry Of Health,[1073] and the latest published national disease surveillance data which contains the nationally notifiable disease list.[1074] The *Public Health Act* includes notification requirements for unknown infectious diseases.[1075] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

178. **Trinidad and Tobago:** The data obtained included information from the Ministry Of Health,[1076] and the *Quarantine Act*.[1077] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

179. **Tunisia:** The data obtained included information from the Ministry Of Health,[1078] the country's published WHO Joint External Evaluation report,[1079] and the *Order of the Minister of Health fixing the list of communicable diseases subject to compulsory notification* which contains the nationally notifiable disease list.[1080] No nationally published disease surveillance data were available.

180. **Turkey:** The data obtained included information from the Ministry Of Health,[1081] the *Communicable Diseases Surveillance And Control Principles Regulation* with annexes which contains the nationally notifiable disease list,[1082] and the latest published national disease surveillance data.[1083] The *List Of Communicable Diseases Based On Notification RG-22/4/2020-31107* includes Public health emergencies of international importance in the disease list and elaborates in guidance for health professionals with a flow chart including diseases of unknown cause.[1084] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

181. **Turkmenistan:** The data obtained included information from the Ministry of Health and Medical Industry,[1085] the country's published WHO Joint External Evaluation report,[1086] and the law on the *Prevention of Infectious Diseases.*[1087] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available.

182. **Tuvalu:** The data obtained included information from the Ministry of Health, Social Welfare and Gender Affairs,[1088] and the *Public Health Regulations.*[1089] The *Regulations* require households to report infectious disease but no nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

183. **Uganda:** The data obtained included information from the Ministry of Health,[1090] the country's published WHO Joint External Evaluation report,[1091] the latest published national disease surveillance data,[1092] and the *Public Health Act* with *Public Health (Control of COVID-19) Rules*.[1093] The National Technical Guidelines for Integrated Disease Surveillance and Response contains the nationally notifiable disease list and includes diseases with an unknown cause.[1094]

184. **Ukraine:** The data obtained included information from the Ministry of Health,[1095] the latest published national disease surveillance data which contains the nationally notifiable disease list,[1096] and the law *On Protection of Population against Infectious Diseases*.[1097] The Ukraine completed a WHO Joint External Evaluation in December 2021, but there has not been a published report as of 31 December 2022.[300]

185. **United Arab Emirates:** The data obtained included information from the Ministry of Health and Prevention,[1098] the country's published WHO Joint External Evaluation report,[1099] the latest published national disease surveillance data,[1100] and the *Public Health* law.[1101] The *Prevention of Communicable Diseases* law, which contains the nationally notifiable disease list, includes unusual and emerging diseases, but only after they have been identified and specified by the government.[1102]

186. **United Kingdom:** The data obtained included information from the Department of Health & Social Care,[1103] the latest published national disease surveillance data,[1104] and the *Public Health (Control of Disease) Act*.[1105] The nationally notifiable disease list has an add on requirement to report "other significant disease" but does not specify new diseases or unknown pathogens,[1106] and this is not on the list itself in legislation.[1107] The UK operates a horizon scanning monitoring system which publishes reports on incidents of public health significance, including novel pathogens and diseases.[1108] The UK operates several syndromic surveillance systems and publishes weekly data reports.[1109] There is a foresight report on infectious diseases which mentions novel pathogens.[1110] There is a laboratory diagnostic guide for

laboratories that mentions new diseases without further guidance.[1111] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

187. **United States of America:** The data obtained included information from the Centers for Disease Control and Prevention,[1112] the country's published WHO Joint External Evaluation report,[1113] the National Notifiable Diseases Surveillance System,[1114] the nationally notifiable disease list,[1115] the latest published national disease surveillance data,[1116] and the *Public Health Service Act*.[1117] The Public Health Emergency Preparedness and Response Capabilities report includes mention of novel viruses and emerging diseases for health professionals and laboratories but does not provide detailed guidance.[1118]

188. **Uruguay:** The data obtained included information from the Ministry of Public Health,[1119] and the latest published national disease surveillance data.[1120] The Decree 41/012,[1121] and the nationally notifiable list of diseases,[1122] include significant public health events or diseases but do not specify further. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

189. **Uzbekistan:** The data obtained included information from the Ministry of Public Health,[1123] the law on *State Sanitary Supervision*,[1124] and decision *On Additional Measures To Prevent The Spread Of Infectious Diseases In The Republic Of Uzbekistan*.[1125] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

190. **Vanuatu:** The data obtained included information from the Ministry of Health,[1126] the *Public Health Act* which contains the nationally notifiable disease list.[1127] No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

191. **Venezuela:** The data obtained included information from the Ministry of Popular Power for Health,[1128] the National Institute of Hygiene,[1129] the *Organic Health Law*,[1130] and published article on national epidemiological surveillance.[1131] No complete nationally notifiable disease list was publicly accessible. No nationally published disease surveillance data were available. No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

192. **Vietnam:** The data obtained included information from the Ministry of Health,[1132] the country's published WHO Joint External Evaluation report,[1133] and the National Institute of Hygiene and Epidemiology.[1134] The *Prevention and Control of Infectious Diseases* law, which contains the nationally notifiable disease list, includes unknown diseases.[1135] No nationally published disease surveillance data were available.

193. **Yemen:** The data obtained included information from the Ministry of Public Health and Population,[1136] the latest National Primary Health Care Strategy,[1137] the decree *Regarding the regulations of the Ministry of Public Health and Population*,[1138] and the latest published national disease surveillance data.[1139] No complete nationally notifiable disease list was publicly accessible, but a publication on the disease surveillance system states the number of diseases under surveillance.[1140] No Joint External Evaluation has been undertaken as of 31 December 2022.[300]

194. **Zambia:** The data obtained included information from the Ministry of Health,[1141] the country's published WHO Joint External Evaluation report,[1142] the Zambia National Public Health Institute,[1143] the latest published national disease surveillance data,[1144] the *Public Health Act* which contains the nationally notifiable disease list,[1145] and *The Public Health (Notifiable Infectious Disease) (Declaration) Notice*.[1146]

195. **Zimbabwe:** The data obtained included information from the Ministry of Health and Child Care,[1147] and the country's published WHO Joint External Evaluation report.[1148] The *Public Health Act*, which contains the nationally notifiable disease list, includes diseases of public health importance outside of the list itself and elaborates that this can relate to any infectious disease or symptom which could lead to adverse community health effects.[1149] No nationally published disease surveillance data were available.

Appendix C: Chapter Two - Additional Results

C.1 Data Availability and Location

Out of the 195 countries included in the study, 176 (90.3%) had national public health laws that met the search criteria enacted and accessible as of 31 December 2022. As shown in Table S2, this varied by WHO region, from 35/47 (74.5%) in the African Region (AFRO) to 100% of the 54 countries in the European Region and 35 countries in the Region of the Americas. A total of 136/195 countries (69.7%) had a full nationally notifiable disease list publicly accessible, with a further 22 (11.3%) only specifying the number of diseases under surveillance. The availability of a full nationally notifiable disease list varied by WHO region, from 23/47 (48.9%) in the African Region to 46/54 (85.2%) in the European Region. From the 136 countries with a full nationally notifiable disease list available, 58 (42.6%) had the list present in national legislation, 33 (24.3%) had it present in a publicly accessible government publication, 24 (17.6%) had it included in a national disease surveillance data report, 18 (13.2%) had it published on a dedicated government webpage, and 3 (2.2%) had the list present in a published peer-reviewed article.

Table S2. Summary of public data by World Bank Income Strata and WHO region for the 195 included countries. WHO: World Health Organisation; AFRO: African Region; AMRO: Region of the Americas; EMRO: Eastern Mediterranean Region; EURO: European Region; SEARO: South-East Asian Region; WPRO: Western Pacific Region.

Category	Number of Countries	Public Health Law Enacted and Publicly Accessible	Full Nationally Notifiable Disease List Publicly Available	Only the Number of Nationally Notifiable Diseases Available	National Disease Surveillance Data Published and Available	Completed and Published WHO Joint External Evaluation
	n	n (%)	n (%)	n (%)	n (%)	n (%)
World Bank Income Strata*						
High	58	57 (98.3)	54 (93.1)	1 (1.7)	48 (82.8)	23 (39.7)
Upper Middle	53	51 (96.2)	35 (66.0)	9 (17.0)	34 (64.2)	21 (39.6)
Lower Middle	54	47 (87.0)	30 (55.6)	9 (16.7)	29 (53.7)	36 (66.7)
Low	27	18 (66.7)	15 (55.6)	3 (11.1)	11 (40.7)	24 (88.9)
WHO Region						
AFRO	47	35 (74.5)	23 (48.9)	8 (17.0)	20 (42.6)	45 (95.7)
AMRO	35	35 (100)	28 (80.0)	0 (0)	22 (62.9)	2 (5.7)
EMRO	21	17 (81.0)	15 (71.4)	4 (19.0)	14 (66.7)	17 (81.0)
EURO	54	54 (100)	46 (85.2)	5 (9.3)	45 (83.3)	17 (31.5)
SEARO	11	10 (90.9)	7 (63.6)	2 (18.2)	7 (63.6)	8 (72.7)
WPRO	27	25 (92.6)	17 (63.0)	3 (11.1)	14 (51.9)	15 (55.6)
Global	195	176 (90.3)	136 (69.7)	22 (11.3)	122 (62.6)	104 (53.3)

* Data from the World Bank based on 2021 reported Gross National Income;[119] unavailable for Venezuela, Cook Islands and Niue

Out of the 195 countries included, a total of 122 (62.6%) had nationally published disease surveillance data available. This varied by WHO region, from 20/47 (42.6%) in the African Region to 45/54 (83.3%) in the European Region, as seen in Table S2. A cut-off of 31 December 2022 was used for national disease surveillance data. This was the latest date covered by the surveillance period in the population and not necessarily the date of data publication.

The latest published data date ranged from 31 December 2007 to 31 December 2022, with a median date of 23 July 2022 (Interquartile Range: 10 Jan 2020 - 22 December 2022). From the 122 countries with published national disease surveillance data, 38 (31.1%) countries had data from within the last month of the cut-off date (1 - 31 December 2022), 72 (59.0%) countries had surveillance data within the last year (31 December 2021 - 31 December 2022),

92 (75.4%) had surveillance data published within the last three years (31 December 2019 - 31 December 2022), and 109 (89.3%) had surveillance data within the last five years (31 December 2017 - 31 December 2022).

A total of 104/195 countries (53.3%) had a complete and published WHO Joint External Evaluation as of 31 December 2022. This varied by WHO region, as seen in Table S2, from 2/35 (5.7%) in the Region of the Americas to 45/47 (95.7%) in the African Region. Out of the 195 countries included, 142 (72.8%) had the frequency of data analysis carried out internally at the national level specified in available resources. From these, 27 (19.0%) stated their country conducted daily data analysis, and 102 (71.8%) specified weekly analysis.
Out of the 195 countries included, 55 (28.2%) stated they publicly released analysed data at least on a weekly basis.

C.2 Additional Predictors of Novel Disease Consideration

Novel disease consideration was compared against the 2021 GHSI and SPAR. This included one specific indicator in each that explicitly asked about unknown pathogens, the full text of which is available in Appendix A. For GHSI Question 2.1.1b on whether countries had a strategy for testing for novel pathogens, 41/67 countries (61.2%) which met some GHSI criteria had evidence of novel disease consideration compared with 32/128 (25.0%) that did not meet the GHSI criteria, with the relationship significant with X^2 (df = 1, N = 195) = 23.08, p-value = <0.0001. For SPAR Capacity 4.4 on whether the country has access to laboratories to identify unknown pathogens, 34/52 countries (65.4%) self-reporting “yes” had publicly available evidence of novel pathogen consideration, compared with 36/132 countries (27.3%) that reported “no.” This association was significant with X^2 (df = 1, N = 184) = 21.40, p-value = <0.0001.

For the WHO African Region, 8/8 (100%) of the countries with novel disease surveillance had adopted the Integrated Disease Surveillance and Response (IDSR) Strategy, compared with 36/39 (92.3%) of countries without evidence of novel disease surveillance, giving a p-value 0.42. Overall, 4/8 (50.0%) of countries with novel disease surveillance had IDSR training in >90% of districts between 2015-2017, compared with 9/35 (25.7%) in countries without novel disease surveillance, with a p-value of 0.18.

Other predictors for any evidence of novel disease consideration in four resources (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines) are shown in Table S3.

Table S3. Predictors of any evidence of novel disease consideration in four resources (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines) vs no evidence using logistic regression. GDP: Gross Domestic Product; USD: United States Dollar; COVID-19: Coronavirus Disease 2019; OR: Odds Ratio; CI: Confidence Interval.

Predictor	**Countries**	**Novel Disease Consideration**	
	N	**OR (95% CI)**	***p*-value**
2021 Human Development Index	189	1.05 (1.03 - 1.08)	<0.0001
Latest Surveillance Data Release, As of the 31 December 2022			
Within Last Month	38	Referent	-
Within Last Year	34	0.66 (0.25 - 1.70)	0.39
Within Last Three Years	20	0.64 (0.21 - 1.94)	0.42
Within Last 15 Years	30	0.22 (0.076 - 0.61)	0.0043
No Public Data Available	73	0.073 (0.026 - 0.19)	<0.0001
Surveillance Data Release Frequency, as Stated			
At Least Weekly	55	Referent	-
At Least Quarterly	26	0.67 (0.26 - 1.73)	0.40
Annually or Variably	41	0.33 (0.14 - 0.75)	0.0096
No Public Data	73	0.080 (0.031 - 0.19)	<0.0001
Stated Internal Data Analysis Frequency			
Daily	27	Referent	-
Weekly	102	0.19 (0.069 - 0.48)	0.00065
Less than Weekly	13	0.11 (0.019 - 0.45)	0.0044
Unknown	53	0.13 (0.041 - 0.35)	0.00012
Event-based Surveillance System			
None	106	Referent	-
Yes, No Daily Analysis	71	2.40 (1.27 - 4.59)	0.0073
Yes, Daily Analysis	18	10.24 (3.35 - 38.61)	0.00013
COVID-19 Data Publicly Released			
No	49	Referent	-
Yes	146	3.47 (1.63 - 8.10)	0.0021
Number of Nationally Notifiable Diseases	158	1.04 (1.03 - 1.06)	<0.0001

Evidence of novel disease consideration varied by national score on a range of different indicators in the WHO Joint External Evaluation for the 104 countries who had one completed and published, as shown in Figure S1.

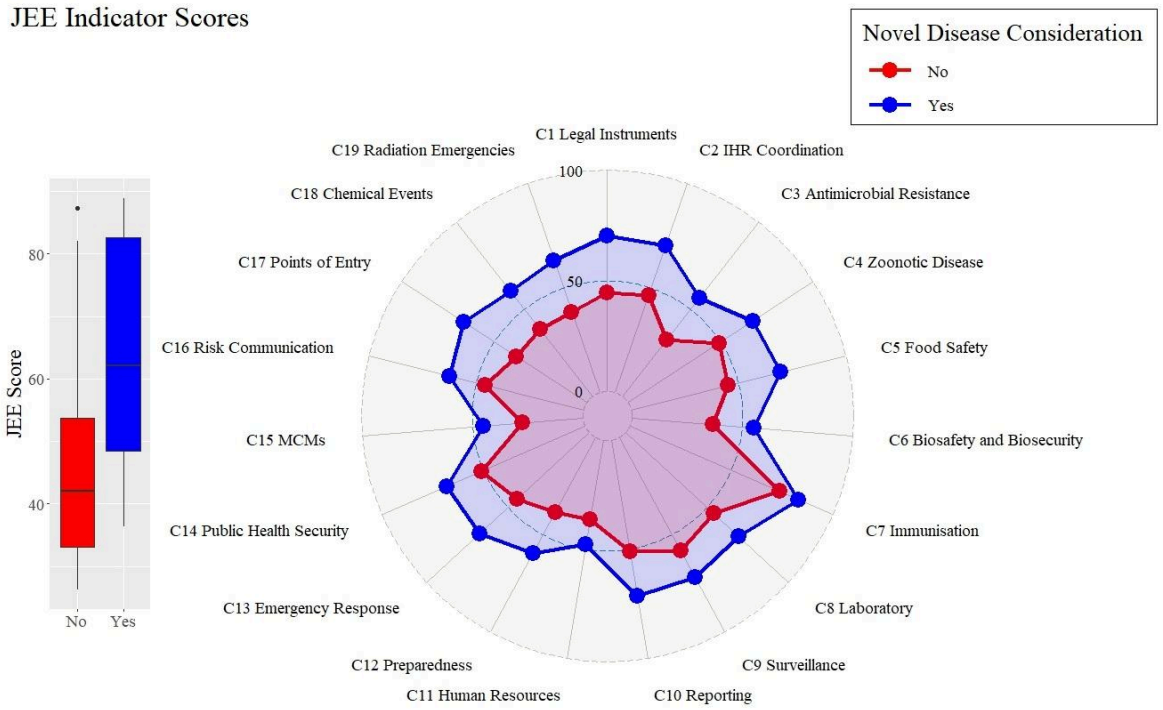


Figure S1. Novel disease consideration in any of the four examined resource types investigated (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines) compared against WHO Joint External Evaluation for 104 countries with data available. Novel disease consideration in one or more resource types in blue with no evidence of consideration in red. WHO: World Health Organisation; JEE: Joint External Evaluation; IHR: International Health Regulations; MCMs: Medical Countermeasures.

Overall, 73/195 countries (37.4%) had any evidence of novel disease consideration in the four resource types investigated, with the global distribution shown in Figure S2.

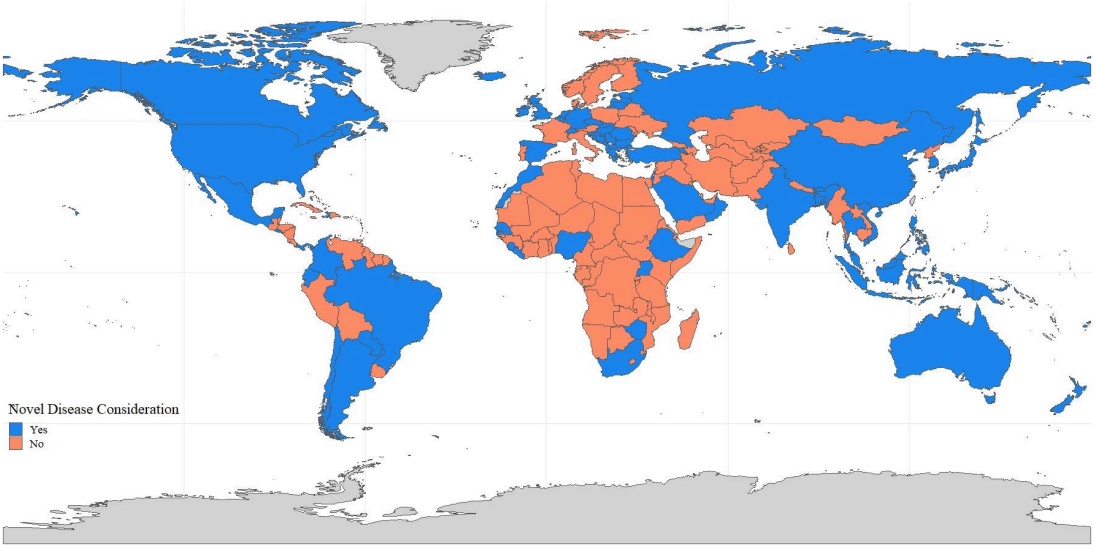


Figure S2. Global map depicting evidence of novel disease inclusion in any of the four resource types investigated (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines) for 195 examined countries. Evidence of novel disease consideration in one or more of the four resources is shown in blue, while countries with no evidence are depicted in red.

Multiple metrics examined were shown to be highly correlated with one another, with linear regressions of various indices shown in Figure S3.

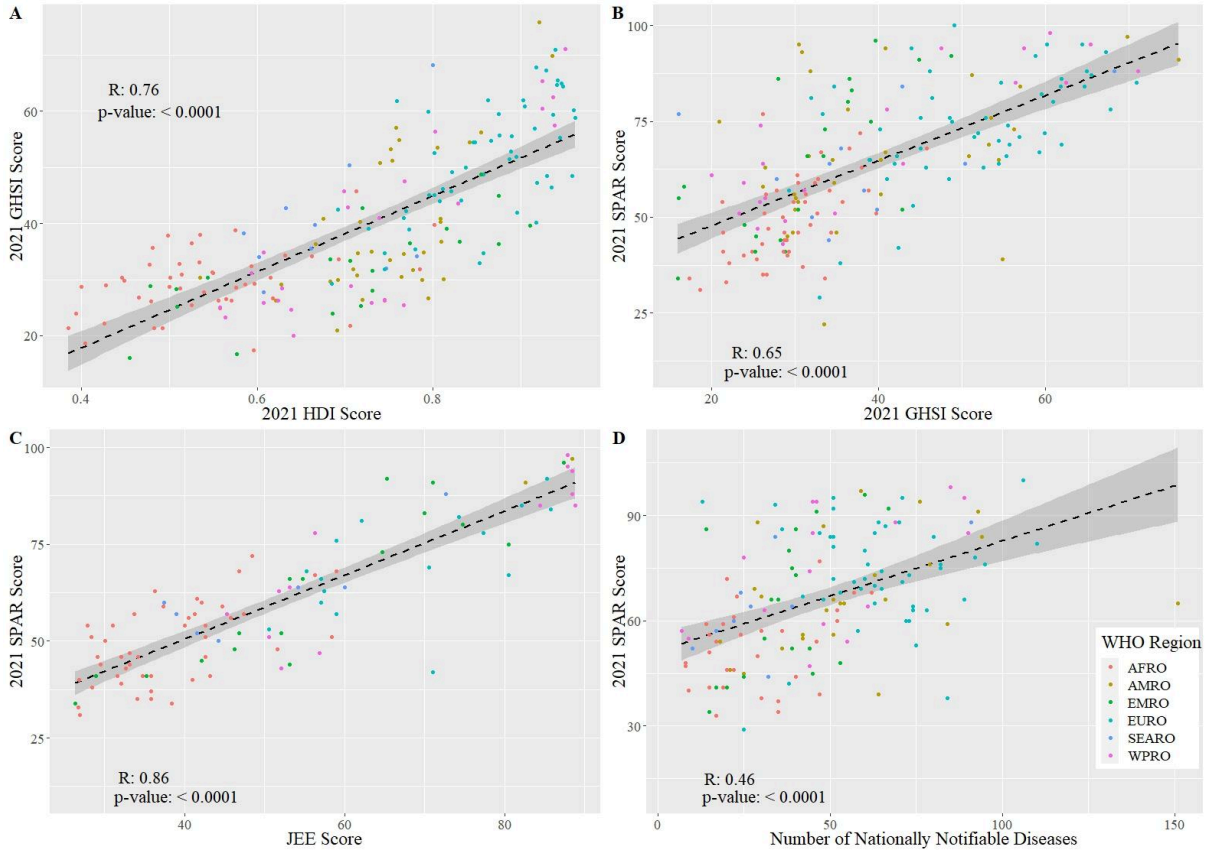


Figure S3. Linear regression and correlation scores for various indices. A: 2021 total GHSI score vs 2021 HDI score for 189 countries with both available; B: 2021 SPAR score vs 2021 GHSI score for 184 countries with both available; C: 2021 SPAR Score vs JEE score for 102 countries with both available; D: 2021 SPAR Score vs the total number of nationally notifiable diseases for 148 countries with both available.

C.3 Ordinal Logistic Regression

An ordinal logistic regression on the same predictor variables as in Table 2.2 in the main paper was carried out. The ordinal logistic regression assumes the odds of each category are proportional, meaning the relationship between moving between a country having two or more resources with evidence of novel disease consideration vs lower categories (zero or one resource with evidence) is the same as the odds of a country having three or more resources with evidence of novel disease consideration vs lower categories (zero, one or two resources with evidence). The ordinal logistic regression results are shown in Table S4.

Table S4. Predictors of any evidence of novel disease consideration in four resources (public health law, the national notifiable disease list, health professional guidelines, and laboratory guidelines) using ordinal logistic regression. WHO: World Health Organisation; AFRO: African Region; AMRO: Region of the Americas; EMRO: Eastern Mediterranean Region; EURO: European Region; SEARO: South-East Asian Region; WPRO: Western Pacific Region; USD: United States Dollar; SARS-CoV-1: Severe acute respiratory syndrome coronavirus 1; MERS: Middle East Respiratory Syndrome; GHSI: Global Health Security Index; SPAR: State Party Self-Assessment Annual Reporting; OR: Odds Ratio; CI: Confidence interval.

Predictor	Novel Disease Consideration		
	n/N (%)	OR (95% CI)	*p*-value
World Bank Income Strata*			
High	30/58 (51.7)	Referent	-
Upper Middle	24/53 (45.3)	0.99 (0.48 - 2.02)	0.98
Lower Middle	15/54 (27.8)	0.34 (0.16 - 0.71)	0.0050
Low	4/27 (14.8)	0.17 (0.046 - 0.50)	0.0029
WHO Region			
EURO	30/54 (55.6)	Referent	-
AFRO	8/47 (17.0)	0.17 (0.066 - 0.41)	0.00015
AMRO	13/35 (37.1)	0.59 (0.25 - 1.34)	0.21
EMRO	3/21 (14.3)	0.13 (0.028 - 0.43)	0.0023
SEARO	6/11 (54.5)	0.68 (0.21 - 2.09)	0.51
WPRO	13/27 (48.1)	0.77 (0.32 - 1.81)	0.55
Health expenditure, $1000 USD per capita[†]	71/186 (38.2)	1.28 (1.10 - 1.50)	0.00097
National Disease Surveillance Data Available			
Yes	64/122 (52.5)	8.06 (3.89 - 18.59)	<0.0001
No	9/73 (12.3)	Referent	-
Completed and Published WHO Joint External Evaluation			
Yes	37/104 (35.6)	0.86 (0.49 - 1.51)	0.59
No	36/91 (39.6)	Referent	-
WHO Joint External Evaluation Score	37/104 (35.6)	1.07 (1.04 - 1.10)	<0.0001
Total Recorded Outbreaks[‡]	73/194 (37.6)	1.03 (0.99 - 1.06)	0.095
One or more cases of SARS-CoV-1, MERS, or Nipah Virus			
Yes	27/43 (62.8)	3.43 (1.81 - 6.52)	0.00016
No	46/152 (30.3)	Referent	-
2021 GHSI Score	73/195 (37.4)	1.10 (1.07 - 1.12)	<0.0001
2021 SPAR Score[§]	70/184 (38.0)	1.06 (1.04 - 1.08)	<0.0001

***** Data from the World Bank based on 2021 reported Gross National Income;[119] unavailable for Venezuela, Cook Islands and Niue

[†] Data from the World Bank based on latest national health expenditure estimates from 2019;[120] unavailable for Albania, Cook Islands, Libya, Liechtenstein, Niue, North Korea, Somalia, Syria and Yemen

[‡] Data based on a global outbreak dataset January 1996 - March 2022;[121] unavailable for Turkmenistan

[§] Data based on country self-reports submitted as of 31 December 2022;[111] unavailable for Andorra, Barbados, Brunei, Grenada, Israel, Marshall Islands, Nauru, Niue, Papua New Guinea, Trinidad and Tobago, and Turkey

Appendix D: Chapter Four - Estimating Prevalence

The following R code provides a simple estimate of SARS-CoV-2 infection prevalence on 20 April 2020 from a doubling time of 3.8 days without public health interventions.[228]

```
# Function to calculate the future prevalence of SARS-CoV-2 infection
future_prevalence <- function(initial_cases, doubling_time, start_date, target_date,
disease_duration) {
    # Convert dates to date objects
    start_date <- as.Date(start_date, format = "%Y-%m-%d")
    target_date <- as.Date(target_date, format = "%Y-%m-%d")
     # Calculate the number of days between start_date and target_date
    days_passed <- as.numeric(difftime(target_date, start_date, units = "days"))
     # Calculate the number of doubling periods
    doubling_periods <- days_passed / doubling_time
     # Calculate the cumulative incidence
    cumulative_incidence <- initial_cases * (2 ^ doubling_periods)
     # Calculate the number of recovered cases
    recovered_start <- start_date + disease_duration
    days_since_recovery <- as.numeric(difftime(target_date, recovered_start,
   units = "days"))

    if (days_since_recovery > 0) {
     doubling_periods_since_recovery <- days_since_recovery / doubling_time
     recovered_cases <- initial_cases * (2 ^ doubling_periods_since_recovery)
    } else {
     recovered_cases <- 0
    }

    # Calculate the prevalence (active cases)
    prevalence <- cumulative_incidence - recovered_cases
     return(prevalence)
   }

# Parameters
initial_cases <- 1                    #Initial number of cases known in UK[196]
doubling_time <- 3.8                  # Doubling time of epidemic in days[228]
start_date <- "2020-01-23"            #First known case in the UK[196]
target_date <- "2020-04-20"           #First sampling test point
disease_duration <- 9.3               #Infectious period (days) for SARS-CoV-2[172]
UK_population <- 67081000             #UK 2020 population from World Bank[1150]

predicted_prevalence <- future_prevalence(initial_cases, doubling_time, start_date,
target_date, disease_duration)
Prev_prop <- predicted_prevalence/UK_population
cat("Predicted prevalence on", target_date, "is", round(Prev_prop*100,digits=2),"%")
```

Predicted prevalence on 2020-04-20 is 11.39 %