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IntechOpen Series
Infectious Diseases, Volume 39

Current Topics in Viral Outbreaks

Edited by Alfonso J. Rodriguez-Morales

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Published in London, United Kingdom

Current Topics in Viral Outbreaks
http://dx.doi.org/10.5772/intechopen.111301
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First published in London, United Kingdom, 2025 by IntechOpen
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British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Current Topics in Viral Outbreaks
Edited by Alfonso J. Rodriguez-Morales
p. cm.

This title is part of the Infectious Diseases Book Series, Volume 39
Topic: Viral Infectious Diseases
Series Editor: Alfonso J. Rodriguez-Morales
Topic Editor: Shailendra K. Saxena

Print ISBN 978-0-85466-333-0
Online ISBN 978-0-85466-332-3
eBook (PDF) ISBN 978-0-85466-394-1
ISSN 2631-6188

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IntechOpen Book Series

Infectious Diseases

Volume 39

Aims and Scope of the Series

This series will provide a comprehensive overview of recent research trends in various Infectious Diseases (as per the most recent Baltimore classification). Topics will include general overviews of infections, immunopathology, diagnosis, treatment, epidemiology, etiology, and current clinical recommendations for managing infectious diseases. Ongoing issues, recent advances, and future diagnostic approaches and therapeutic strategies will also be discussed. This book series will focus on various aspects and properties of infectious diseases whose deep understanding is essential for safeguarding the human race from losing resources and economies due to pathogens.

Meet the Series and Volume Editor

Dr. Alfonso J. Rodriguez-Morales received his MD from Universidad Central de Venezuela in Caracas and his MSc in Protozoology/Parasitology from Universidad de Los Andes in Trujillo, Venezuela. He received his Diploma in Tropical Medicine & Hygiene (DTM&H) from Universidad Peruana Cayetano Heredia, Lima, Peru, and the University of Alabama at Birmingham, Alabama, USA. He also holds a DipEd. Dr. Rodriguez-Morales is a fellow of the Royal Society for Tropical Medicine & Hygiene (FRSTMH), London, United Kingdom; of the Faculty of Travel Medicine (FFTM) of the Royal College of Physicians and Surgeons of Glasgow (RCPSG), Glasgow, Scotland, United Kingdom; of the American College of Epidemiology (FACE), USA; and the International Society for Antimicrobial Chemotherapy (FISAC). He has a HonDSc from Universidad Privada Franz Tamayo (UniFranz), Cochabamba, Bolivia. He is the President of the Latin American Society for Travel Medicine (SLAMVI) (2023-2025) and the Past President of the Colombian Infectious Diseases Association (2021–2023). He is a member of the Council (2020-2026) of the International Society for Infectious Diseases (ISID). He is a senior researcher at Colciencias (2015–2027) and a professor at the Fundación Universitaria Autónoma de las Américas in Pereira, Risaralda, Colombia, as well as at the Universidad Científica del Sur (UCSUR) in Lima, Peru. He is a visiting professor at multiple national and international universities.

Contents

Preface

Viral outbreaks continue to pose a significant threat to global health, security, and development. From localized epidemics to devastating pandemics, the emergence and re-emergence of viral pathogens such as Ebola, Zika, influenza, mpox, and most recently SARS-CoV-2, highlight the critical need for preparedness, surveillance, and rapid response. *Current Topics in Viral Outbreaks* brings together leading researchers, clinicians, and public health experts to examine the multifaceted dimensions of contemporary viral threats. This volume synthesizes cutting-edge knowledge across virology, epidemiology, clinical management, One Health, and global health policy. It also addresses the ecological, social, and technological factors that drive viral emergence and transmission in an increasingly interconnected world. This book aims to inform future strategies for mitigating outbreaks and improving resilience by exploring past lessons and present challenges. We hope it serves as both a reference and an inspiration for continued vigilance, research, and collaboration in confronting viral diseases worldwide.

Considering these issues, this book presents research and clinical topics related to what has been learned about viral outbreaks, particularly on viral zoonoses, SARS-CoV-2/COVID-19, and Mpox, among others. The book's eight chapters are organized into two major sections: "Epidemiology" and "Clinical Aspects, Diagnostics and Miscellaneous".

Commissioning this book by IntechOpen is partly related to my long commitment to tropical and emerging diseases, mainly vector-borne, zoonotic, and neglected tropical diseases. I am a member of the Council of the International Society for Infectious Diseases (ISID) (2020-2026) and Past President of the Colombian Association of Infectious Diseases (*Asociación Colombiana de Infectología*, ACIN) (2021-2023), as well as of the Committee on Tropical Medicine, Zoonoses and Travel Medicine of the ACIN. During 2020, I founded the Latin American Network of Research on COVID-19 (LANCOVID). LANCOVID has contributed to the research on SARS-CoV-2/COVID-19 in Latin America. Since 2024, I have been a member of the World Health Organization (WHO) Guideline Development Group for Clinical Management of post-COVID-19 condition (2024-2025), and a Member of the Expert Panel for Developing the Rapid Guideline on Mpox of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID).

Following the same philosophy we used for my ten previous books with IntechOpen, this book is not intended to be an exhaustive compilation. Research on SARS-CoV-2/COVID-19 has become highly dynamic and rapidly evolving, necessitating consultation with the most recent available evidence for informed diagnostic and treatment decisions.

I would like to extend a very special thank you to IntechOpen, particularly Elvira Baumgartner, Publishing Process Manager, and Dajana Pemac, Commissioning Editor, for the opportunity to edit this fascinating and important book, as well as for their constant support.

I would like to take a moment to dedicate this book to my beloved family, who are spread geographically across Venezuela, Chile, and Colombia, although physically distant, they are close in our hearts (Aurora, Alfonso José, Alejandro, and Andrea, the neurologist). Katterine, my loving wife, soul, and passion, makes every day special and cares about every aspect of my life. She is also my perfect life mate, travel companion, and, more importantly, in life's journey. I love her more than anything, and I am happy to have her lovely existence with our canine kids, Antonieta and Jazz. I love you more and more every day.

I would also like to thank my friends and undergraduate and postgraduate students in Colombia, Venezuela, and Latin America. In 2019, I began working at the Fundación Universitaria Autónoma de las Américas, Pereira, Risaralda, Colombia, a new "home" that provides me with support and trust in my new endeavors in research and teaching. I would like to extend special thanks to Drs. Maria Monica Murillo, our dean at the Faculty of Medicine, and our former School of Medicine Director, Dr. Jaime Cardona-Ospina, a long-time friend and fellow, is now a PhD student in the United States of America. I would also like to thank the significant support of Dr. Jose Antonio Suárez, "Tony" (Venezuela/Panama), who has been very special to me over time, and to my friend Dr. Alberto Paniz-Mondolfi (Venezuela/USA).

Finally, I hope our readers enjoy this publication as much as I enjoyed putting it together with my talented and knowledgeable collaborators.

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Section 1

Epidemiology

Chapter 1

Introductory Chapter: Global Challenges of Viral Outbreaks

Alfonso J. Rodriguez-Morales

1. Introduction

In recent decades, viral outbreaks have emerged as the most formidable challenges to global health, security, and socioeconomic stability. From the HIV/AIDS pandemic to the catastrophic impact of Ebola in West Africa [1–10], Zika in the Americas [11–20], and most recently, the global crisis caused by SARS-CoV-2 [21–30], the frequency and scale of viral epidemics and pandemics have intensified [31–34]. This trend reflects a convergence of factors—ecological disruption, globalization, urbanization, climate change, and increased human-animal interface—that have accelerated the emergence and re-emergence of viral pathogens [35–46].

The interconnectedness of today's world means that localized outbreaks can rapidly evolve into global threats. The COVID-19 pandemic starkly illustrated how quickly a novel virus can cross borders, overwhelm healthcare systems, and disrupt economies and societies at every level [26, 47–55]. It also highlighted critical weaknesses in preparedness, surveillance, global coordination, and the deep inequities in access to healthcare and vaccines. These lessons remain urgently relevant as new outbreaks continue to emerge, including those caused by known threats like influenza [56–65] and monkeypox [66–77], and potential threats such as novel coronaviruses and arboviruses [32, 33, 78–83].

Viral outbreaks do not occur in a vacuum. They intersect with political, social, environmental, and economic dynamics influencing their spread and our responses. Misinformation, vaccine hesitancy, and inadequate health infrastructure can exacerbate outbreaks, while geopolitical tensions may hinder effective global collaboration. Understanding the complexity of these challenges requires a multidisciplinary and One Health approach, recognizing the interconnectedness of human, animal, and environmental health [84, 85].

This book, *Current Topics in Viral Outbreaks*, brings together experts from various fields to explore the multifaceted challenges of viral diseases. This introductory chapter lays the foundation for the following subsequent discussions by framing the global context of viral outbreaks and emphasizing the urgent need for resilient surveillance systems, robust public health infrastructure, scientific innovation, and international solidarity. As we move deeper into the twenty-first century, future outbreaks are inevitable. The question is not if, but when and how prepared we will be.

This volume aims to inform, challenge, and inspire collective action toward a more resilient and equitable global health future by comprehensively examining past and ongoing outbreaks, emerging pathogens, and strategic responses [86–88].

2. Epidemiology of viral outbreaks

The epidemiology of viral outbreaks encompasses the study of the distribution, determinants, and dynamics of viral diseases within populations. Understanding these epidemiological patterns is crucial for identifying at-risk populations, implementing effective control measures, and preventing future outbreaks. Viral epidemics and pandemics often exhibit complex transmission dynamics that depend on pathogen characteristics, host factors, environmental influences, and social determinants of health [89, 90].

Viral pathogens can be transmitted through a variety of routes, including respiratory droplets (e.g., influenza, SARS-CoV-2), fecal-oral spread (e.g., hepatitis A, norovirus), vector-borne transmission (e.g., dengue, Zika, chikungunya), and direct contact with infected bodily fluids (e.g., Ebola, HIV) [91–100]. The reproductive number (R_0), incubation period, and duration of infectiousness are key epidemiological parameters that inform the potential for spread and the design of public health interventions [101–104].

Globalization and rapid urbanization have amplified the potential for viruses to spread across continents within days. Air travel, trade, and population displacement due to conflict or climate change have created conditions favorable to the rapid dissemination of infectious agents. Additionally, ecological changes such as deforestation and encroachment into wildlife habitats have increased the risk of zoonotic spillover events, where viruses cross species barriers to infect humans, as seen with the Nipah virus [105–114], SARS, MERS, and SARS-CoV-2 [115, 116].

Modern molecular epidemiology and genomic surveillance have revolutionized the investigation of outbreaks. Whole-genome sequencing enables the real-time tracking of viral evolution, allowing for the identification of new variants and transmission chains with unprecedented speed and accuracy. During the COVID-19 pandemic, genomic data played a crucial role in monitoring the emergence of variants of concern, informing vaccine updates, and shaping public health policy. However, disparities in sequencing capacity between high-income and low- and middle-income countries remain a significant challenge [117, 118].

The burden of viral outbreaks is often unevenly distributed, with marginalized communities experiencing higher morbidity and mortality due to limited access to healthcare, poor living conditions, and systemic inequities. Epidemiological analyses must therefore incorporate a socioecological perspective, recognizing how structural determinants influence vulnerability and resilience [119].

Effective epidemiological surveillance systems, both passive and active, are essential for detecting and responding to outbreaks. Integrating human and animal health surveillance through a One Health framework enhances early warning capabilities and facilitates coordinated action [120–131]. As viral threats evolve, so too must our epidemiological tools and approaches, ensuring they are agile, inclusive, and globally coordinated [132].

3. Main clinical impact of emerging viral diseases

Emerging viral diseases pose significant and multifaceted clinical challenges (**Table 1**). As new or previously controlled viruses reappear in human populations, they frequently present with variable clinical manifestations, ranging from asymptomatic infection to severe, life-threatening illness. Viral virulence, host immunity,

System	COVID-19 (SARS-CoV-2)	Ebola virus disease (EVD)	Zika virus	Dengue virus	Chikungunya virus
General	Fever, fatigue, malaise	Sudden high fever, fatigue	Mild fever, rash	High fever, headache, retro-orbital pain	High fever, rash, fatigue
Respiratory	Cough, dyspnea, hypoxia; ARDS in severe cases	Cough is rare, secondary to systemic involvement	Mild or absent respiratory symptoms	Generally absent	Mild cough occasionally
Cardiovascular	Tachycardia, myocarditis, thromboembolism	Hypotension, shock in late stages	Rare fetal bradycardia	Hypotension, plasma leakage (severe dengue)	Rare arrhythmias, possible myocarditis
Neurological	Headache, anosmia, encephalopathy, stroke	Confusion, delirium in severe stages	Guillain-Barré syndrome, microcephaly and congenital Zika syndrome (fetus)	Encephalopathy, seizures (rare)	Polyarthritis, neuroinflammation (rare)
Gastrointestinal	Diarrhea, nausea, vomiting	Profuse vomiting, diarrhea, and abdominal pain	Nausea and vomiting occasionally	Nausea, vomiting, abdominal pain	Nausea, vomiting, mild diarrhea
Musculoskeletal	Myalgia, fatigue	Severe myalgia, weakness	Arthralgia (mild)	Myalgia, arthralgia	Severe arthralgia, chronic arthritis-like symptoms
Dermatologic	Rash (infrequent), chilblain-like lesions	Petechiae, bleeding, rash in late stages	Maculopapular rash, conjunctivitis	Maculopapular rash, bleeding in severe forms	Maculopapular rash, facial flushing
Hematologic	Lymphopenia, elevated D-dimer, and thrombocytopenia	Leukopenia, thrombocytopenia, elevated liver enzymes	Mild leukopenia	Leukopenia, thrombocytopenia, hemoconcentration	Mild thrombocytopenia, lymphopenia
Imaging	Chest CT: ground-glass opacities, consolidation	Nonspecific; GI imaging may show fluid accumulation	Neuroimaging: calcifications (congenital cases)	Chest X-ray: pleural effusion (severe cases)	Joint imaging: synovitis, tenosynovitis (rare)

Table 1.
Main clinical, laboratory, and imaging findings of some selected emerging viral infections by organs and systems.

comorbidities, age, and access to healthcare influence the clinical spectrum. These infections strain clinical services and often reveal systemic gaps in healthcare preparedness and response [133, 134].

Respiratory viruses, such as novel influenza strains, SARS-CoV-1, MERS-CoV, and SARS-CoV-2, commonly cause acute respiratory syndromes of varying severity. These can lead to pneumonia, acute respiratory distress syndrome (ARDS), and multiorgan failure. COVID-19, in particular, underscored the diverse clinical impact of respiratory viruses, ranging from mild symptoms to severe complications including thrombosis, cardiac injury, neurological sequelae, and prolonged post-viral syndromes (e.g., Long COVID) [135–138]. The burden on intensive care units and the need for ventilatory support further complicated the clinical response during pandemic peaks [139, 140].

Hemorrhagic fevers, such as those caused by Ebola, Marburg, Lassa, and Crimean-Congo hemorrhagic fever viruses, are often associated with high case fatality rates. These diseases are characterized by fever, vascular leakage, coagulopathy, and multi-organ dysfunction. Managing these infections requires level isolation and supportive care, which are often unavailable in resource-limited settings where such viruses are endemic or emerging. Clinical outcomes are frequently poor without early intervention, underscoring the importance of rapid diagnosis and supportive therapy [141, 142].

Arboviral infections—including dengue, chikungunya, Zika, and yellow fever—demonstrate the broad clinical impact of emerging viruses. Dengue can cause a range of outcomes, from a mild febrile illness to severe dengue hemorrhagic fever and shock syndrome. Zika virus, though often mild in adults, has devastating effects on fetal development, causing congenital Zika syndrome with microcephaly and other neurodevelopmental anomalies. Chikungunya is primarily associated with febrile polyarthritis, which can persist chronically and impair quality of life [42, 44, 46, 89, 143].

Neurotropic viruses, such as West Nile [144–153], Japanese encephalitis, and rabies [154–162] can cause central nervous system involvement, including encephalitis, meningitis, or paralytic syndromes. These diseases often result in long-term neurological impairment or death, particularly in vulnerable populations such as the elderly, immunocompromised, and unvaccinated individuals [163].

Beyond the acute illness, emerging viral diseases often leave a lasting clinical and societal impact. Post-infectious sequelae—neurological, cardiovascular, renal, and psychiatric—are increasingly recognized. In outbreaks, healthcare systems face the challenge of managing acutely ill patients while also addressing their long-term follow-up and rehabilitation needs. The psychological impact on both patients and healthcare workers, including post-traumatic stress and burnout, is substantial and often underestimated [164–167].

Moreover, co-infections with endemic pathogens (e.g., malaria, HIV, tuberculosis) can alter clinical outcomes, complicating diagnosis and treatment. For example, co-infection with HIV and viral hepatitis or SARS-CoV-2 may exacerbate disease severity and alter therapeutic responses. Immunocompromised individuals are particularly susceptible to prolonged viral shedding, atypical presentations, and poor prognosis [168, 169].

The lack of specific antiviral therapies for many pathogens further challenges the clinical management of emerging viral diseases. In most cases, treatment is supportive, relying on fluid management, respiratory support, and symptomatic care. While vaccines offer a powerful tool for prevention, access and acceptance remain uneven

globally. The rapid development of vaccines against COVID-19 has demonstrated the potential of modern biotechnology, however, other emerging threats lack effective preventive or therapeutic options [170].

In sum, the clinical impact of emerging viral diseases is broad and profound. These infections not only cause direct morbidity and mortality but also strain healthcare systems, exacerbate inequities, and create long-term public health burdens. A comprehensive clinical response requires preparedness, training, resource allocation, and investment in research to understand pathogenesis better, develop targeted therapies, and build resilient health systems [171].

4. Research challenges on viral outbreaks

The research landscape surrounding viral outbreaks is complex, rapidly evolving, and challenging to navigate. As emerging and re-emerging viruses continue to pose global threats, timely and robust research is crucial for informing public health responses, therapeutic development, and vaccine strategies. However, logistical, ethical, political, and infrastructural obstacles often hinder conducting high-quality research during outbreaks [81–83, 89, 90, 135].

One major challenge is the unpredictability of outbreaks, which makes it difficult to establish pre-approved research protocols and mobilize resources swiftly. By the time research frameworks are deployed, the outbreak may already be in decline, leading to missed opportunities for data collection. This was evident in multiple Ebola outbreaks, where delays in initiating clinical trials limited the ability to assess promising therapeutics in real time [87, 88, 102, 170].

Another barrier is limited infrastructure in affected regions, particularly in low- and middle-income countries where many outbreaks originate. Weak laboratory capacity, lack of trained personnel, and insufficient funding impede rapid diagnostics, genomic surveillance, and clinical research. These gaps contribute to delayed pathogen identification, poor case characterization, and limited data sharing [37, 79, 90, 137].

Ethical considerations also present significant challenges. In the urgency of an outbreak, conducting randomized controlled trials while ensuring informed consent, equitable access, and protection of vulnerable populations requires careful planning and robust oversight. Balancing scientific rigor with the humanitarian imperative to offer potentially life-saving interventions remains a delicate endeavor [103, 135, 164, 170, 172].

Data sharing and coordination are often fragmented across national and institutional lines. Proprietary restrictions, a lack of open-access platforms, and geopolitical tensions can delay the dissemination of critical epidemiological and genomic data. The COVID-19 pandemic underscored both the benefits of open science and the consequences of data silos [44, 102, 115, 139, 169].

Finally, funding sustainability for outbreak-related research is typically reactive and short term. Investment tends to surge during crises and decline afterward, undermining long-term preparedness and innovation [173].

To overcome these challenges, the global research community must prioritize collaborative networks, equitable funding mechanisms, adaptable ethical frameworks, and the development of agile research infrastructure. Strengthening these areas is crucial to generating timely, actionable evidence and ensuring a coordinated response to current and future viral threats.

5. Conclusions

Viral outbreaks have become an enduring global threat, testing the resilience of health systems, scientific infrastructure, and international cooperation. From COVID-19 to Ebola, Zika, dengue, and chikungunya, the emergence and re-emergence of viral pathogens continue to reveal critical vulnerabilities in surveillance, clinical response, and research readiness. As outlined in this chapter, the global challenges posed by these outbreaks are multidimensional, spanning epidemiological complexity, clinical burden, research constraints, and the need for coordinated global action.

Epidemiologically, emerging viruses exploit a convergence of risk factors including ecological disruption, urbanization, globalization, and increased human-animal interactions. Rapid spread across borders—often facilitated by asymptomatic carriers and inadequate surveillance—demands vigilant monitoring systems and international data sharing. A One Health approach, integrating human, animal, and environmental health, is fundamental to anticipating and mitigating the next outbreak.

Clinically, these viral diseases exhibit diverse manifestations, affecting multiple organ systems and resulting in acute and long-term health consequences. The differential severity across populations underscores the influence of social determinants, comorbidities, and health inequities. The lack of specific treatments for many emerging viral diseases means that supportive care, early diagnosis, and prevention remain the cornerstones of effective management.

Research during outbreaks, though indispensable, faces significant obstacles. These include unpredictable timing, ethical complexities, logistical constraints, and inequities in funding and infrastructure. The experience of COVID-19 illustrated the critical value of agile research frameworks, open science, and public-private partnerships—but also highlighted enduring disparities between nations in terms of capacity and access.

The global community must shift from reactive crisis management to proactive preparedness. This includes investing in surveillance and laboratory networks, strengthening healthcare delivery systems, fostering transdisciplinary research, and ensuring equitable access to diagnostics, therapeutics, and vaccines. Resilience requires scientific innovation, trust, transparency, and solidarity across borders.

This introductory chapter sets the stage for a deeper exploration of the topics in viral outbreaks addressed in the rest of this book. We aim to contribute to a more coordinated, evidence-based, and equitable global strategy to confront current and future viral threats by advancing our understanding of the underlying challenges and responses.

Acknowledgements

The author acknowledges the use of Grammarly Premium® Academic, Microsoft® Editor/Copilot for language polishing of the manuscript. Additionally, EndNote is used for managing references, and Microsoft Word is used for writing.

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Chapter 2

Perspective Chapter: Viral Zoonoses – Pathways and Mechanisms

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Abstract

Viral zoonoses are infectious diseases caused by viruses that are naturally transmitted from non-human vertebrates to humans. Many viruses of animal origin, such as the influenza viruses, dengue virus, ebola virus, SARS coronavirus and others, are significant public health, economy and biodefence concerns. Hence, there is a substantial interest in addressing the various aspects of viral zoonosis, including the detection of viral reservoirs, developing an understanding of the role of hosts, vectors and environment in the emergence of viral zoonoses, vector ecology, molecular mechanisms underlying the host jump and establishing the modes of transmission. This chapter describes the current understanding of the pathways and mechanisms involved in the emergence of viral zoonoses and their impact on developing strategies for controlling zoonotic diseases.

Keywords: viral zoonoses, reservoir host, spillover, virus transmission, virus evolution, one health approach, global virome project

1. Introduction

Zoonotic viruses have caused fatal diseases in humans over the years (**Table 1**). Further, zoonotic viruses also continue to emerge and re-emerge, causing epidemics, pandemics and many of them establishing themselves as endemic in different regions across the globe (**Figure 1**) [2, 3]. Only a few of the zoonotic viruses have been controlled through measures such as vaccination and surveillance and others sustain to cause periodic outbreaks, leading to significant socioeconomic impact (**Figure 1**, **Table 1**). The phenomenon of viral zoonosis is, therefore, a serious public health concern. In addition, it affects the trade of animal-based products and some of the zoonotic viruses are also listed among the Biodefense Pathogens by the National Institute of Allergy and Infectious Diseases (NIAID) [4]. Despite challenges, particularly posed by the vast diversity of viral and animal species, studies have addressed various aspects of viral zoonosis. Research has shown that the animal viruses cross species boundaries through spillover events driven by various factors [5, 6]. Modes of transmission and transmission

S. no.	Virus	Genus and family	Fatality rate (%)	Incidence
1.	Avian influenza A virus	Influenza virus A; *Orthomyxoviridae*	Conjunctivitis: 0%; Pneumonia and ARDS: H5N1 (52%), H7N7 (1%), H7N9 (30%)	H5N1:45/year; H7N2:<1/year; H7N7:<1/year; H7N9: 1–800/year; H9N2: 4/year
2.	Chandipura virus (CHPV)	Vesiculovirus; Rhabdoviridae	56–75% in children	Sporadic cases (2009, 2010)
3.	Chikungunya virus (CHIKV)	Alphavirus, Togaviridae	<0.1% and 0.6% in 60+ years	Outbreaks in different parts of the world
4.	Crimean-Congo hemorrhagic fever virus (CCHFV)	Orthonairovirus; Bunyaviridae	10–40%	Sporadic incidences: 10–500/year
5.	Dengue virus (DENV)	Flavivirus; Flaviviridae	95–99% mild: 0%; 0.5–5% Dengue hemorrhagic fever(DHF): Treated <1% and Untreated 10–20%; Dengue shock syndrome: Treated 1–10% and Untreated 20–50%.	Large outbreaks with appx. 400 million cases, worldwide each year
6.	Eastern equine encephalitis virus (EEEV)	Alphavirus, Togaviridae	Febrile illness (0%); Encephalitis (30%)	Less than 100 cases per year
7.	Ebolavirus (EBOV)	Ebolavirus, Filoviridae	Hemorrhagic fever (50%)	Sporadic outbreaks
8.	Hantaan virus (HTNV)	OrthoHantavirus; Bunyaviridae	Hemorrhagic fever with renal syndrome (10%)	100,000–200,000 cases each year
9.	Hendra virus (HeV)	Henipavirus; paramyxoviridae	Encephalitis (50%)	Sporadic
10.	Human influenza A virus	Influenzavirus A, Orthomyxoviridae	Hospitalized elders (>65 years): 11%; Children (Reye syndrome): 21%; Children Encephalitis: 18%	Seasonal (winter)
11.	Human influenza B virus	Influenzavirus B, Orthomyxoviridae	Hospitalized elders (>65 years): 11%	Seasonal (winter)
12.	Human SARS coronavirus 1 (SARS-CoV)	Betacoronavirus; Coronaviridae	Acute respiratory distress syndrome (ARDS) 9%; Elderly >65 years, 50%	Sporadic, Outbreak 2002–2004
13.	Human SARS coronavirus 2 (SARS-CoV)	Betacoronavirus; Coronaviridae	Acute respiratory distress syndrome (ARDS) 9%; Elderly >65 years, 50%	Endemic
14.	Japanese Encephalitis Virus (JEV)	Orthoflavivirus; Flaviviridae	Asymptomatic (0%); Encephalitis (30%)	Outbreak
15.	Junin arenavirus (JUNV)	Arenavirus; Arenaviridae	Argentine hemorrhagic fever (15–30%)	Sporadic
16.	Lake Victoria marburgvirus (MARV)	Marburgvirus; Filoviridae	Marburgvirus disease; Hemorrhagic fever (80%)	Outbreak
17.	Lassa virus (LASV)	Arenavirus; Arenaviridae	Mild, Asymptomatic (0%); Hemorrhagic fever (15–20%)	Outbreak

S. no.	Virus	Genus and family	Fatality rate (%)	Incidence
18.	Lymphocytic choriomeningitis virus (LCMV)	Arenavirus; Arenaviridae	Mild (0%); Aseptic meningitis (<1%); Encephalitis (<1%); Meningoencephalitis (< 1%); Congenital: abortion, malformations (30%)	Sporadic, Outbreak
19.	Macacine alphavirus 1 (McHV-1) CDC	Simplexvirus, Herpesviridae	Acute transverse myelitis (0%); Encephalitis (80%)	<1/year
20.	Machupo virus (MACV)	Arenavirus; Arenaviridae	Bolivian hemorrhagic fever: 5–30%	Sporadic
21.	MERS Coronavirus	Betacoronavirus; Coronaviridae	MERS (35%)	Sporadic
22.	Mokola virus (MOKV), Shimoni bat virus, Lagos bat virus	Lyssavirus, Rhabdoviridae	Fatal encephalitis (100%)	Sporadic
23.	Monkeypox virus (MPXV)	Orthopoxvirus	Clade I: 3.6%; Clade IIB: 0.03%	(clade IIA) Sporadic, Outbreak; (clade IIB) Endemic
24.	Murray valley encephalitis virus (MEV)	Flavivirus; Flaviviridae	Encephalitis (25%)	Sporadic
25.	Nipah Virus (NiV)	Henipavirus; paramyxoviridae	Encephalitis (40–75%)	Sporadic
26.	Puumala virus (PUUV)	Hantavirus, Bunyavirus	Nephropathia Epidemica (<0.1%)	Sporadic
27.	Rabies virus (RABV) Aravan virus (ARAV), Duvenhage virus (DUVV), Australian bat Lyssavirus (ABLV), European bat lyssavirus (EBLV), Irkut virus (IRKV), Khujand virus (KHUV)	Lyssavirus, Rhabdoviridae	Fatal encephalitis (100%)	Sporadic
28.	Rift valley fever virus (RVF)	Phlebovirus, Phenuiviridae	mild, fever (0%); Retinitis ()%); Meningoencephalitis (0.1%); Acute hepatitis with hemorrhage (50%).	Sporadic, Outbreaks
29.	Saint Louise encephalitis virus (SLEV)	Flavivirus; Flaviviridae	Aseptic meningitis (0%); Encephalitis Elder (10–20%)	Sporadic, Outbreaks
30.	Sin Nombre virus (SNV)	OrthoHantavirus; Bunyaviridae	Hantavirus cardiopulmonary syndrome (HCPS): 35%; Acute respiratory distress syndrome (ARDS): 90%	Sporadic
31.	Tick-borne encephalitis virus (TBEV)	Flavivirus; Flaviviridae	Tick-borne encephalitis: 0.5–2%	Sporadic
32.	Venezuelan equine encephalitis virus (VEEV)	Alphavirus, Togaviridae	Mild: 0%; Encephalitis: <1%	Sporadic
33.	West Nile virus (WNV)	Flavivirus; Flaviviridae	Asymptomatic or mild fever (0%); Encephalitis (10%)	Sporadic, Outbreaks

S. no.	Virus	Genus and family	Fatality rate (%)	Incidence
34.	Yellow fever virus	Flavivirus; Flaviviridae	Hemorrhagic fever (20–50%)	Sporadic, Outbreaks
35.	Zika virus	Flavivirus; Flaviviridae	Asymptomatic mild fever (0%); Guillain-Barre syndrome: 5%; Congenital infection (Microcephaly and other malformations): 64%	Sporadic, Outbreaks

Table 1.
List of zoonotic viruses with public health burden.

Figure 1.
"en"—endemic, an outbreak is referred to as endemic *when there is a continuous occurrence of the disease in a population at an approximately constant and predictable level. "ep"—epidemic, when the disease spreads rapidly to a large number of people as compared to an endemic rate, it is referred to as an* epidemic. *"pn"—pandemic, a worldwide epidemic is referred to as a* pandemic. *Emerging infectious diseases (EID) are those that have shown an increase in incidence numbers in the past two decades, becoming a local or global public health problem. Re-emerging infectious diseases (REID) are those which were once major health problems then declined significantly but are re-occurring and causing major health problems recently [1]. "v"—approved vaccines: Avian Influenza A virus: Influenza vaccine, (Inactivated); Dengue virus: Dengue vaccine, (Qdenga, Dengvaxia, TV003/TV005); Ebola virus: Ebola vaccine, (VSV- and Adenovirus-vectored vaccines); Human Influenza A virus: (Attenuated or Inactivated) [Influenza A(H1N1, H3N2) and B/Victoria); Human Influenza B virus: (Attenuated or Inactivated) [Influenza A(H1N1, H3N2) and B/Victoria); Chikungunya virus: CHIKV vaccine (Attenuated); Japanese Encephalitis Virus: JEV vaccine; Monkeypox virus: Monkeypox vaccine, (attenuated or Inactivated): Rabies virus: Rabies vaccine (Inactivated); Yellow fever virus: Yellow fever vaccine (attenuated). SARS coronavirus 2: Covaxin (Inactivated), Covilo (Inactivated), CoronaVac (Inactivated), Spikevax (RNA), Comirnaty (RNA), Convidecia (non-replicating viral vector), Jcovden (non-replicating viral vector), Vaxzevria (non-replicating viral vector), Covishield (non-replicating viral vector).* Eastern equine encephalitis virus infection likely provides life-long immunity against re-infection.

capability of many zoonotic viruses have also been established. Upon a host jump from an animal, humans are the dead-end hosts for some of the zoonotic viruses, such as the rabies virus, because they either have no or have limited capability of human-to-human transmission or transmission to any other host. Whereas, some zoonotic viruses, such as the SARS coronaviruses, zika virus, dengue virus and some others, have undergone

genomic evolution and have emerged as new human viruses, capable of either human-to-human transmission (horizontal or vertical) or transmission through vectors [7]. The following sections describe our current understanding of reservoirs of the zoonotic viruses, various spillover pathways, molecular mechanisms involved in the evolution of viral zoonoses and a discussion on future perspectives for control of viral zoonoses.

2. Reservoirs of zoonotic viruses

A Pathogen reservoir is defined as the host where the infectious agent normally lives, multiplies, endemically circulates, co-evolves, is maintained permanently and gets transmitted to the target population [8, 9]. A pathogen could have multiple reservoirs and the disease may or may not be manifestated in the reservoir host. In addition to the reservoirs, intermediate/amplifying host, spillover host, susceptible host and non-susceptible host have also been described for some of the viruses, such as the SARS coronaviruses [9]. Appropriate methods to establish the existence of a reservoir, clear identification of the reservoir(s), determination of host range and managing them can help prevent fatal epidemics [10, 11]. Control of many diseases such as Ebola virus infection and rabies has been hampered due to an incomplete understanding of their reservoirs [12–14].

Majority of the zoonotic viruses belong to the families, *Flaviviridae*, *Arenaviridae*, *Rhabdoviridae*, *Bunyaviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Filoviridae* and *Togaviridae* (**Table 1**) [15]. Among their identified animal reservoirs, mammalian (Rodentia, Primates, Chiroptera, Cetartiodactyla, Perissodactyla, Carnivora, Diprotodontia, Artiodactyla) and avian species (Passeriformes, Anseriformes, Galliformes) represent the majority [15–17]. Such studies have suggested that the risk of cross-species transmission for the emergence of viral zoonosis arises not only from the animal species that are evolutionarily closer to humans but also from distant groups like rodents, bats, ungulates and birds (**Figure 2**) [16, 18–21]. Two popular hypotheses that have emerged, providing explanations for the noted patterns, are the "special reservoirs hypothesis" and "reservoir richness hypothesis". According to the "Special reservoir hypothesis," a high ecological overlap, due to the domestication of certain evolutionarily distant animal groups such as rodents and ungulates, provides opportunities for pathogen transmission to humans. Whereas, according to the "species richness hypothesis," existence of a large number of certain animal species such as rodents and bats relative to other mammalian groups, is responsible for their high representation among the virus reservoirs [22].

In addition to identifying the animal taxa that are more likely to maintain and/or transmit zoonotic viruses and recognizing any patterns in the relationship between viruses and their reservoirs, it is also of interest to understand if the zoonotic ability is determined by traits of the virus alone or the reservoir also has an active role. Studies have emerged on viral immunity in bats, which are reservoirs for many significant viruses. Bats are noted to have an immune system that tolerates many zoonotic viruses including the SARS-CoV 1 and 2, Ebola virus and Hendra virus [23]. Role of bat immune response on virus replication, clearance and persistence is not fully understood. However, an interesting hypothesis that has been proposed is that, distinct selective pressures in bats might have triggered the evolution of viral accessory proteins. It is conceived based on observations such as the observed truncations in the accessory proteins of zoonotic viruses hosted by bats [24]. Most of the viral accessory proteins are poorly characterized but have an important role in pathogenesis.

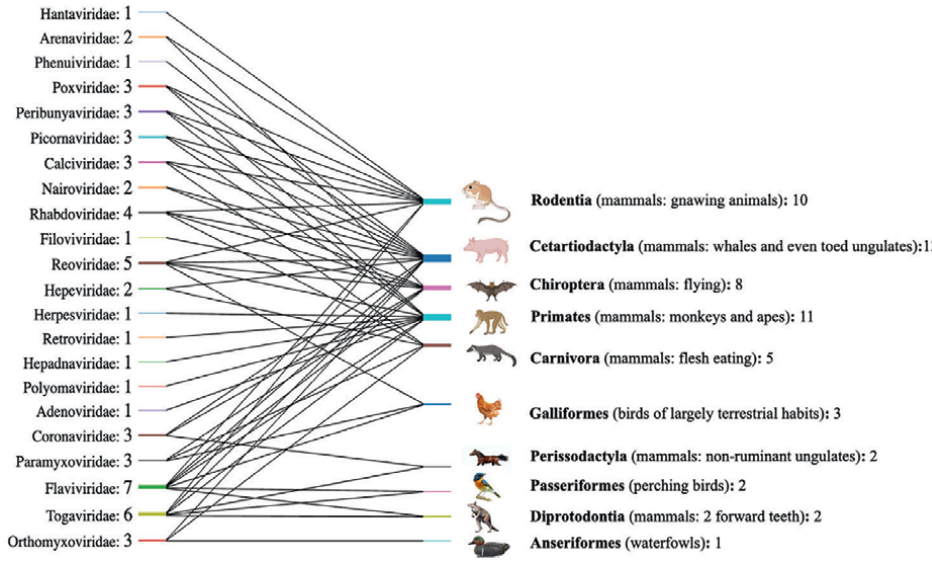

Figure 2.
Reservoirs of zoonotic viruses. Viral families are listed on the left and the numbers placed next to them represent the number of their (identified) reservoir animal species. The reservoir species are listed on the right and the numbers placed next to them represent the number of viral families associated with them.

Hence, the investigation of viral immunity in the animal reservoirs and it is impact on zoonoses could improve our understanding of the principles of viral zoonosis.

Involvement of vectors and maintenance of zoonoses by sylvatic cycle is another aspect of interest. Arthropods, including ticks, flies and mosquitoes, are important transmission vectors. Arboviruses such as Chikungunya virus, Dengue virus, zika virus and Yellow fever virus have originated in non-human primates. In the natural forest habitats of non-human primates, these viruses are transmitted by mosquitos from infected to naïve animals in what is known as a sylvatic transmission cycle [25]. Sylvatic cycles allow the maintenance of arboviruses and possibly also provide opportunities for development of new viral strains. The sylvatic transmission cycle can "spill over" to urban transmission cycle when humans infected in forests spread the virus among people *via* urban mosquitoes [26–29]. Chikungunya virus, dengue virus and zika virus are fully adapted to urban cycles and do not really require sylvatic cycle for their maintenance [30]. Yellow fever virus is maintained by sylvatic (jungle), intermediate (savannah) and urban cycles (**Figure 3**). An amplification of Yellow fever virus in non-human primates, prior to short-lived outbreaks in urban population has been documented [31]. The *Aedes* or *Haemagogus* species of mosquitoes can transmit the Yellow fever virus to humans or non-human primates by feeding on infected primates (human or non-human). The sylvatic cycle involves virus transmission between non-human primates and species of mosquitoes found in the forest canopy (**Figure 3**). Occasionally, forest mosquitoes can also transmit the virus to humans when humans are working or visiting the forest. In the intermediate (savannah) cycle, in Africa, the virus is transmitted by mosquitoes from non-human primates to humans or from humans to humans when humans are working or living in areas bordering the jungle. In the urban cycle, virus (which is usually brought to the urban areas by a viremic human who was infected in the jungle or savannah) is transmitted between humans and urban mosquitoes, primarily *Aedes aegypti*. Further,

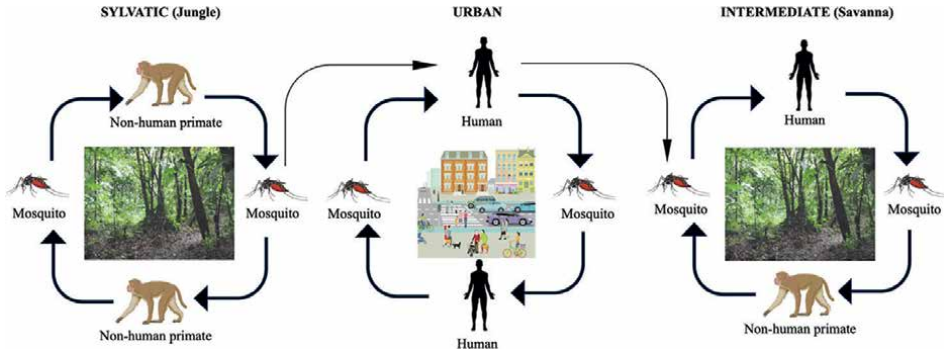

Figure 3.
Sylvatic, urban and intermediate transmission cycles of Yellow fever Virus in Africa.

based on serological analyses, several other zoonotic arboviruses including Mayaro virus, Oropouche virus, O'nyong'nyong virus, Spondweni virus and Lumbo virus are suspected to be maintained in nature by sylvatic cycles [32, 33].

3. Spillover of zoonotic viruses

Spillover is the transmission of a pathogen from a non-human vertebrate host to humans. The six main components in the chain of infection are pathogen, reservoir, portal of exit, mode of transport, portal of entry and susceptible host. In the spillover pathway of viral zoonoses, the virus leaves its animal reservoir through a portal of exit and enters the susceptible host(s), through an appropriate portal of entry, either by a direct or an indirect mode of transmission (**Figure 4**). In some of the pathways, amplifying or intermediate hosts are also involved. For instance, palm civet is identified as the intermediate/amplifying host in the emergence of SARS coronavirus zoonosis. Furthermore, apart from humans, multiple other spillover/susceptible hosts are identified for SARS coronavirus, which include Chinese ferret badger, domestic cat, dog, pig and racoon [34, 35]. Portal of exit refers to the site where the pathogen is localized and can be transmitted, such as body fluids, urine, fecal material, mucus, oral cavity and skin. Whereas, portal of entry refers to the site through which the pathogen can enter the susceptible host and gain access to the tissues in which it can multiply. Infectious agents may use the same or separate sites for exit and entry. For example, influenza virus uses the respiratory tract as the portal of exit as well as the portal of entry, whereas the polio virus, which is transmitted through "fecal-oral" route, uses separate portals for exit and entry. The direct transmission of zoonotic viruses involves contact of humans with the infected animals. The indirect transmission refers to the transfer of the infectious agent from the reservoir to a host either by suspended air particles (airborne transmission), inanimate objects (transmission by vehicles) or animate vectors (vector-borne transmission). Vehicles for indirect transmission of an infectious virus include food, water and biological samples such as blood and fomites. Vectors such as mosquitoes, ticks and fleas can transmit infectious viruses. Vectors may be mechanical carriers, support growth or cause changes in the infectious agents. Modes of transmission of various zoonotic viruses are summarized in **Table 2**.

Humans are regularly exposed to many potentially infectious pathogens of animal origin. However, most of them cannot spillover and cause disease in humans. It is a

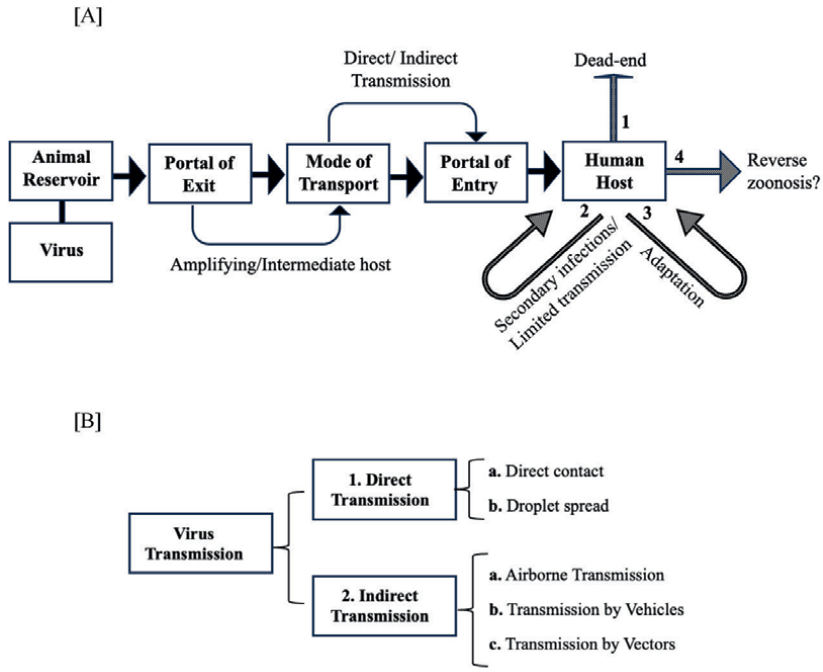

Figure 4.
[A] Chain of infection in viral zoonoses. [B] Types of transmission of zoonotic viruses.

Mode of transmission	Virus
Direct: (i) direct contact or droplet spread	Influenza A virus, Ebola virus, Hendra virus (animal bite), Macacine alphavirus 1 (animal bite), Mokola virus (animal bite), Monkeypox virus, Nipah virus (animal bite), Rabies virus (animal bite), Sin Nombre virus (aerosols/bites/scratches)
Direct: (ii) vertical transmission	Zika virus
Indirect: (i) Airborne virus is carried by dust or droplet nuclei suspended in air (dried residues, smaller than five microns)	SARS coronaviruses, influenza A virus
Indirect: (ii) Vehicle-borne virus is transmitted through agents such as food, water, biologic products and fomites.	Hantaan virus (urine, saliva), Junin arenavirus (fomites), Lake Victoria marburgvirus (fomites), Lassa virus (urine, fomites), Lymphocytic choriomeningitis virus (fomites), Machupo virus (fomites), Puumala virus (urine, saliva), Sin Nombre virus (urine, saliva), influenza A virus (contaminated surfaces)
Indirect: (iii) Vector-borne virus is transmitted by vectors such as mosquitoes, fleas and ticks. Vector may provide just a mechanical means or may support growth or change in the virus.	Chandipura virus, Chikungunya virus, Dengue virus, Japanese Encephalitis virus, Crimean-Congo hemorrhagic fever virus, Eastern equine encephalitis virus, Murray valley encephalitis virus, O'nyong-nyong virus, Rift valley fever virus, Saint Louis encephalitis virus, Tick-borne encephalitis virus, Venezuelan equine encephalitis virus, West Nile virus, Yellow fever virus, zika virus

Table 2.
Transmission modes of zoonotic viruses.

relatively rare event because the probability of a spillover is determined by the ability of the virus to cross a series of nonlinear and dynamic barriers outside – as well as within the spillover host [36, 37]. Among the factors that determine the probability of a successful spillover are the distribution and density of the reservoir; prevalence of the zoonotic virus and intensity of the infection; release, survival and spread of the virus; human encounter and a productive infection of the spillover host [5]. In addition to the inherent properties of the reservoir, the virus and the spillover host, spillover events are also driven by factors such as anthropogenic activities, demography, climate, environment and ecological conditions [5, 38, 39]. For instance, in the year 1999, a severe outbreak of the Nipah virus took place in Malaysia, due to virus spillover from bats to pigs [40]. Ecological changes such as encroachment into the bat habitat encouraging an encounter, intensification of pig farming resulting in a high density of hosts and international trade leading to the spread of infection are suggested as significant contributors [41, 42]. Climate and environmental changes are speculated to have played a role in the emergence of hantavirus and spillover of Hendravirus. An escalation in the population of rodents as a result of environmental changes is suggested to have led to the emergence of hantavirus in 1993 [43]. Similarly, climatic changes are predicted to have contributed to southward movement of black flying fox, a reservoir of Hendra virus, leading to the spillover of the virus into the southern horse populations, which subsequently infected humans [44, 45]. New opportunities for spillover and emergence of zoonoses are created by anthropogenic activities as well. Rapid urbanization an increase in populations residing in low-quality and crowded dwellings have promoted the emergence and spread of arboviruses such as chikungunya virus, dengue virus and zika virus. Studies have shown that the vectors for these viruses, *Aedes aegypti* and *Aedes albopictus* mosquitoes, are well adapted to urban areas [46–48]. Dense and highly connected urban areas are transmission centers for rapid spread of viruses such as SARS coronaviruses and influenza viruses [49, 50]. Further, demographic changes are also noted for contribution to the emergence of viral zoonoses. It has been postulated that globally aging populations and aging immune landscapes heighten the risk for spillover [51–53].

4. Molecular mechanisms in the emergence of zoonoses

There are multiple ways by which humans can encounter animal viruses. However, encounter alone is not sufficient for them to emerge as human pathogens. For the establishment of the disease, a virus also needs to be internalized, replicate efficiently in the human host, evade host defenses and be disseminated [54, 55]. A zoonotic virus becomes a threat to the human population when it has evolved the capacity for human-to-human transmission and is able to spread beyond the spillover zone. SARS-CoV 2 and H1N1 influenza virus (swine flu) are examples of zoonotic viruses, which have overcome multiple molecular and physiological barriers for a successful jump to humans, acquired the ability of human-to-human transmission and have spread beyond the spillover zone, causing pandemics. Rabies, caused by a Lyssavirus from the Rhabdoviridae family; West Nile encephalitis, caused by a Flavivirus from the Flaviviridae family; and monkeypox, caused by an Orthopoxvirus, are examples of zoonotic viruses with limited or no human-to-human transmission, resulting in outbreaks that are typically endemic in nature. Spillover could also lead to emergence of new human viruses. AIDS (HIV) and smallpox (variola virus) are viral diseases that started as a zoonosis, but the causative viruses eventually evolved into human-only strains.

Virus evolution is an important driver in the emergence of zoonoses and the appearance of novel human viruses. Using the approaches of genomics and comparative genomics, studies have shown that the virus evolution is a continuous process occurring both, before and after the emergence of zoonosis [7]. Under selective pressure, adaptive mutations are rapidly acquired by viruses due to their inherent properties of high mutation rates, short generation times and large populations [56–59]. The mechanisms of viral genome evolution could involve either small but significant changes through point mutations, small deletions and/or insertions or major genome remodeling through events such as recombination and/or reassortment [60, 61]. Alterations in viral genomes are known to influence their adaptation to new host by modulating key features, such as the specificity of receptor binding, dynamics of virus fusion, protein synthesis, immune evasion strategies and transmission efficiency (**Table 3**).

S. no.	Virus	Adaptive mutations
1.	SARS-CoV	i. Insertions in the spike protein
		ii. Modification of nuclear localization signal
		iii. Adaptation of spike protein to human receptor (S479N/487T)
		iv. Deletions in accessory protein (ORF8)
2.	Influenza virus H1N1	i. Adaptation to multiple hosts due to multiple reassortments
		ii. Binding of HA to human receptor, SAa2,6Gal (HA 190D/225D)
		iii. Binding of PB2 subunit of RNA polymerase to host factor, ANP32 for replication (PB2 590S/591R)
		iv. Loss of virulence factor
3.	MERS-CoV	i. Insertion in spike protein, which adds flexibility and aids fusion
		ii. Recombination and spike adaptations among camels
		iii. Modification of nuclear localization signals
		iv. Deletion of accessory protein (ORF 4b del)
4.	EBOLA virus	i. Epistatic effect on fusion
		ii. Persistent infections leading to diversification (GP82V)
5.	Zika virus	i. Bottleneck mutations
		ii. Adaptation to host protease
		iii. Viremia (C 106A, NS1 188V, prM 139N, ENV 473M)
		iv. Virulence factors
		v. Persistent infections leading to diversification
6.	SARS-CoV 2	i. Spike inserts add flexibility and aid fusion
		ii. Modified nuclear localization
		iii. Furin cleavage site on the spike protein
		iv. Trimer stabilization (S 614G)
		v. Nuclear localization signal mutations (N 204R) (S 477N) (S 501Y)
		vi. Receptor affinity and neutralization antibody destabilization (S 484A/K, S 417N/T), (S 452R, S 478K)
		vii. Deletions in accessory protein (ORF8)

Table 3.
Identified adaptive mutations in zoonotic viruses.

Influenza A virus has segmented genomes allowing rapid reassortment of genomic segments among viruses co-infecting a single cell [62]. Reassortments can generate chimeric viruses with mix of genes useful for virus adaptation to multiple hosts [63]. Hence, influenced by cross-species transmissions between humans, pigs and birds, the flu landscape has changed multiple times [64, 65]. The influenza virus that caused the epidemic of 1989, likely originated from an avian reservoir host and descended to another virus, H2N2, which caused an epidemic in 1957. Reassortment of H2N2 and an avian virus resulted in the H3N2 virus, which caused the epidemic in 1968. Along with H3N2 in circulation, H1N1 was reintroduced in the year 1977, and by 1998, a chimera of the three viruses was circulating in pigs, which contained HA, NA and PB1 genes of the human H3N2 origin, M, NP and NS of the swine H1N1 and PA and PB2 of the avian origin virus. Currently circulating influenza virus, H1N1 S-OIV, emerged in humans in the year 2009, as a result of reassortment of an "avian-like" Eurasian H1N1 of swine lineage with the triple reassorted swine virus. Point mutations leading to changes in the receptor-binding pocket of the hemagglutinin protein of the virus have helped its adaptation to the human receptor, SAα2,6Gal [60, 66]. Other adaptive mutations of influenza virus include a substitution in the PB2 protein and truncation in the gene encoding a major virulence factor, PB1-F2.

Ebolavirus infection is characterized by extremely severe symptoms and with more than 40% case fatality rate [67]. It has caused several outbreaks, likely through multiple zoonoses and unknown intermediate hosts, which might have played a role in transmission from its major natural host, bats [67–69]. Many amino acid substitutions in the surface glycoprotein, some with epistatic effects, have emerged during the ebolavirus outbreaks, resulting in an enhanced human cell entry and fusion [70, 71]. These mutations could also promote antibody escape and contribute to increased mortality since glycoprotein is the only surface protein on ebolavirus particles [72, 73]. Ebolavirus persistence and the possibility of latency in immune-privileged sites are also noted [74]. These selective environment conditions could provide unique opportunities for virus evolution [73, 75, 76].

Three of the betacoronaviruses, SARS-CoV, SARS-CoV2 and MERS-CoV likely originated in bats and were passed to humans through intermediate/amplifying hosts. Genome of the SARS-CoV, with horseshoe bat as the reservoir host and palm civets as the intermediate host, harbors six amino acid residues in the Receptor Binding Domain (RBD) of spike protein that are different between human and civet isolates. The human receptor, ACE2, but not the civet ACE2, appears sensitive toward substitution of the residues at positions 479 and 489 in RBD, indicating that the virus evolved through acquired mutations for adaptation to human host. Spike inserts add flexibility and aid fusion (**Table 3**). Genome of the SARS-CoV2 has more than 96% similarity to a bat coronavirus, RaTG13, indicating it is origin in bats. Unlike RaTG13, the SARS-CoV2 genome contains an insertion with furin-like cleavage site in S protein, which could promote virion dissemination. The evolution of SARS-CoV2 continued through the course of its epidemic. Within the first year of the epidemic, spike protein with a mutation D614G which, had an impact on ACE2 binding, appeared as a dominant isolate. The first four significant variants, Alpha, Beta, Gamma and Delta are characterized by spike protein mutations, 501Y, 417N:484K:501Y, 417T:484K:501Y and 452R:478K, respectively. Mutation 501Y imparts a dramatic effect on binding affinity. Whereas, 484K and 417N/T disrupt neutralizing antibody binding. The omicron variant which, is highly infectious and immune evasive as compared to prior variants, contains 15 mutations in RBD and 9 nucleotide insertions in the N-terminus domain in the S protein. It also has adaptive mutation in the N protein, affecting its

nuclear localization and is associated with increased pathogenicity potential [77]. Additionally, large deletions are present in the accessory genes, particularly ORF8 [78, 79]. Emergence of omicron variant which has a significantly different genome sequence and measurably different immune response, indicates that SARS-CoV2 could potentially evolve into various regional subtypes and distinct serotypes with limited cross-protection [75]. Unlike the two SARS coronaviruses, the primary animal reservoir for MERS-CoV is well established, which is the dromedary camels [80]. Further, the receptor for MERS-CoV is Dipeptidyl-peptidase 4 (DPP4), which is different than the other two betacoronaviruses, but similar signatures in the spike protein are observed. Entry of MERS-CoV is gained by spike cleavage-mediated membrane fusion. Bat cellular proteases are compatible with both, MERS-CoV and HKU4-CoV spike proteins but the human protease can mediate the entry of MERS-CoV and not of HKU4-CoV [81]. MERS-CoV spike has an insertion of four amino acids, next to the fusion peptide, which likely contributes to the observed compatibility and generally, MERS-CoV spike is able to adapt to species variation in the receptor DPP4 [82]. Another distinguishing feature of MERS coronavirus is that, unlike the SARS coronaviruses, recombination rates among MERS isolates are high in camels and several independent MERS-CoV zoonoses are predicted [83]. Further, the human transmission of MERS coronavirus is limited, suggesting its constraints on infectivity and low host tolerance [84]. Two distinct clades of MERS coronavirus are found in the Arabian and the North African region and similar to the SARS coronaviruses, deletions in their genes encoding accessory proteins, ORF3, ORF4a and ORF4b have been found in both [24, 85].

Zika virus has shown a geographical directionality of migration with a likely origin in Africa, prior to 1950 and moving sequentially through Asia by 1966, Pacific islands by 2007 and the Americas by 2015 [86]. Over this period, the virus has evolved significantly and is reflected by changes in its epidemiological properties. It began with endemic circulation and mild symptoms but progressed to causing epidemic cycles and manifestations of major neurological complications [87, 88]. Zika virus is transmitted through the mosquito *Aedes aegypti* and sexual and vertical transmissions [89]. Zika virus was first isolated from non-human primates but the early molecular events important for human infection or the reservoirs prior to host jump to humans are not clearly identified [87]. As the virus migrated eastward, several mutations that it has acquired have been identified (**Table 3**). The key changes lie within the envelop protein receptor motif, prM. Substitutions within prM can impact cytotoxicity and tissue tropism [88, 89]. Substitution in prM has been associated with congenital Zika virus syndrome and Guillain-Barre syndrome [89–91]. V473M substitution within the envelop protein is shown to increase viremia [92]. A188V is another important substitution within NS1, which results in enhanced interferon inhibition and increased viral titers in brain [89, 93]. T106A substitution in the capsid protein enhances its cleavage by the viral protease and thus virion maturation and infectivity [94].

5. Conclusion and future perspectives

The pathways and mechanisms underlying the emergence of viral zoonosis involve a complex interplay of viral, host, environmental, climatic and anthropogenic factors. Identification of viral reservoirs, understanding of the spillover principles and pathways and recognition of the transmission modes provide opportunities for developing effective public health interventions and reducing the risk of spillovers.

For instance, an understanding of the spillover principles and molecular evolution of the SARS-CoV 2 proved to be of significant value in the control of COVID 19. Some zoonotic viruses may not require molecular evolution for transmission/spillover into humans. The emergence of the Hantavirus and Hendra virus is likely due to climatic and environmental factors. Similarly, urbanization could have promoted the emergence of arboviruses such as chikungunya virus, dengue virus and Zika virus. The observed changes in the spread and clinical symptoms of monkeypox virus suggest that there is a need to establish clear epidemiological relationships and the chains of transmission [95]. Our current understanding of viral zoonoses has been useful for developing ways to control some of the diseases. The EYE (Eliminate Yellow Fever Epidemics) strategy to eliminate the Yellow fever virus is comprehensive, multi-component and multi-partner. Endemic zoonotic viruses including the influenza virus and the Dengue virus are among the global priority pathogens for the development of vaccines [96]. Rabies is included in WHO's 2021–2030 roadmap for global control of Neglected Tropical Diseases. The framework for the control of viral zoonoses is based on the concept of "One Health." Efforts such as the launch of a global virome project will be beneficial for the control of outbreaks in the future [97–100].

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Chapter 3

Airborne and Surface Transmission of SARS-CoV-2 in Hospital Settings: Evidence from a COVID-19 Dedicated Hospital in India

Anuupama Suchiita, Bidhan Chandra Koner, Lal Chandra and Subash Sonkar

Abstract

This chapter investigates the airborne and surface transmission of SARS-CoV-2 within a dedicated COVID-19 hospital in Delhi, India, during the peak of the pandemic. The study, conducted between July and September 2020, employed air and surface sampling in key hospital areas, including the intensive care unit (ICU), medicine ward, and emergency ward. Air samples were collected at 1- and 3-m distances from patients, while surface swabs were taken from high-touch areas such as patient beds, floors, and nursing stations. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to detect viral RNA. Results confirmed the presence of SARS-CoV-2 in the air up to 3 m from patients, with higher viral loads closer to the source, and widespread surface contamination, particularly on patient beds and floors. The findings highlight the dual transmission pathways of SARS-CoV-2, emphasizing the risks of airborne transmission in poorly ventilated spaces and fomite transmission on frequently touched surfaces. The study underscores the importance of robust infection control measures, including improved ventilation, the use of personal protective equipment (PPE), and regular disinfection of high-contact surfaces. These insights provide critical evidence for refining infection prevention strategies in healthcare settings, particularly in resource-limited environments, to mitigate the spread of SARS-CoV-2 and protect healthcare workers and patients.

Keywords: airborne transmission, surface contamination, SARS-CoV-2, hospital infection control, fomite transmission, ventilation and air filtration, personal protective equipment (PPE), RT-PCR, nosocomial infections, COVID-19 pandemic

1. Introduction

The COVID-19 pandemic, driven by the SARS-CoV-2 virus, has placed immense strain on healthcare systems globally [1]. A key factor in controlling the spread of the virus is

understanding its transmission methods. While SARS-CoV-2 primarily spreads through respiratory droplets, emerging evidence emphasizes the role of airborne transmission [1, 2]. Smaller aerosol particles can linger in the air for extended periods, potentially infecting those who inhale them [3]. Additionally, fomite transmission, where the virus remains viable on surfaces and can infect individuals upon contact, has also been documented. These transmission routes pose significant risks, particularly in hospital settings where COVID-19 patients are concentrated, and healthcare workers are on the front lines [4, 5].

Hospital environments are particularly vulnerable to both airborne and surface transmissions due to the high density of individuals, including severely ill patients who shed large amounts of the virus. This increases the risk of contamination, especially in poorly ventilated areas and on frequently touched surfaces such as door handles, bed rails, and medical equipment. Understanding the dynamics of airborne and surface transmission in these settings is critical for implementing effective infection control measures and safeguarding healthcare workers, patients, and visitors [6–8].

This chapter provides evidence-based insights into the transmission of SARS-CoV-2 through the air and on surfaces within a COVID-19 dedicated hospital in India [9]. The study aims to evaluate the presence of the virus in various areas of the hospital, identify contamination patterns based on patient load and proximity to infected individuals, and discuss the implications of these findings for infection prevention and control strategies [7]. Through these insights, the chapter seeks to enhance the understanding of viral transmission pathways and inform policies to reduce the risk of infection in healthcare environments [10].

2. Background

Global research has extensively studied SARS-CoV-2 transmission since the pandemic began, focusing on both airborne and surface routes. Initially thought to spread mainly through respiratory droplets, studies have shown that smaller aerosols, which remain suspended in the air for long periods, also play a significant role, particularly in enclosed, poorly ventilated spaces like hospitals [11]. Additionally, the virus has been found on high-touch surfaces such as door handles and medical equipment, raising concerns about fomite transmission [12–14].

Hospitals are key environments for studying viral transmission, given their high density of infected patients and healthcare workers. These settings face unique challenges, such as overcrowding, inadequate ventilation, and high patient turnover, all of which increase the risk of airborne and surface transmission [15, 16]. Healthcare workers, often in close contact with COVID-19 patients, are at heightened risk, despite personal protective equipment (PPE) measures [17, 18]. Frequent disinfection is crucial but difficult to maintain in busy areas, making hospitals particularly vulnerable to viral spread [1, 19, 20].

Understanding these transmission dynamics is essential for guiding infection prevention and control (IPC) strategies, especially in resource-limited hospitals. By addressing these challenges, hospitals can better protect healthcare workers and patients, minimizing the risk of SARS-CoV-2 transmission [5].

3. Hospital setting and study design

The study was conducted at Lok Nayak Hospital (LNH) in Delhi, India, a major public hospital designated for COVID-19 treatment. LNH handled both moderate

and severe cases, offering services such as intensive care, emergency treatment, and general medicine. Located in one of the world's most densely populated cities, the hospital experienced high patient turnover, making it ideal for studying airborne and surface transmission of SARS-CoV-2 [9, 21].

The hospital's wards, including the ICU, medicine, and emergency wards, were adapted to accommodate COVID-19 patients. The ICU had advanced ventilation with HEPA filters, while the medicine and emergency wards relied on natural ventilation and air conditioning. This variation allowed for a detailed study of transmission dynamics across different ventilation conditions.

The study, conducted between July 1 and September 25, 2020, focused on sampling air and surfaces in the hospital. Air samples were collected from the medicine ward, ICU, and emergency ward, while surface swabs were taken from patient beds, floors, and nursing stations [22–24]. Air samples were collected at 1- and 3-m distances from patients using a total suspended particulate (TSP) air sampler, while surface samples were collected from frequently touched areas. All samples were tested using RT-PCR to detect SARS-CoV-2 [25–27].

This design enabled a comprehensive investigation into the spread of the virus, providing insights into both airborne and surface transmission risks in a hospital setting.

4. Sampling methods and procedures

Air sampling was conducted using a total suspended particulate (TSP) air sampler, calibrated to national standards. Equipped with PVDF filters (100 nm), the sampler operated at flow rates of 1.5, 16.7, and 27 LPM for 1-hour periods. It was placed at 1- and 3-m distances from patients in the medicine ward and ICU, while in the emergency ward, it was positioned centrally due to transient patient presence. After each sample, the filter was placed in viral transport media (VTM) and transported to the lab for RT-PCR testing. Negative control samples were taken from a green zone to rule out contamination.

Surface sampling targeted high-touch areas in patient beds, ward floors, and nursing stations. Swabs were collected from 2 square feet areas near patient beds and from tables in nursing stations. These samples were also stored in VTM and sent for RT-PCR analysis.

Sampling locations were chosen based on areas with high viral shedding potential, such as near patient beds in the medicine ward (moderate cases) and ICU (severe cases). In the emergency ward, air sampling captured overall contamination due to high patient turnover. Nursing stations were included to assess healthcare worker exposure [17, 20, 21].

Data collection occurred from July 1 to September 25, 2020, during peak COVID-19 activity. Sampling in the medicine ward and ICU focused on patients admitted within the last 48 hours, while in the emergency ward, air samples were taken during high turnover periods. This method allowed for comprehensive analysis of airborne and surface contamination in the hospital [28].

5. Laboratory analysis

Laboratory analysis for detecting SARS-CoV-2 in air and surface samples primarily used reverse-transcriptase polymerase chain reaction (RT-PCR). This method

targeted two genes: the E-gene (for general coronavirus detection) and the RdRp gene (specific to SARS-CoV-2). Air samples, collected using PVDF filters, were processed by extracting RNA, followed by RT-PCR using the STANDARD M nCoV Real-Time Detection kit. Surface swabs were similarly processed, and a cycle threshold (Ct) value below 35 indicated a positive result for SARS-CoV-2.

Although viral culture was not performed due to resource limitations, RT-PCR provided sensitive detection of viral RNA. Quality control measures included using negative controls from COVID-free areas, ensuring accurate sample handling and transport, and employing automated RNA extraction systems to minimize error. The RT-PCR kit used was clinically validated, ensuring reliable detection.

Samples were collected on multiple days from different wards to ensure consistency and reproducibility. These measures confirmed the presence of SARS-CoV-2 in both air and surface samples, ensuring the study's accuracy and reliability.

6. Results

Air sampling across the hospital wards confirmed the presence of SARS-CoV-2 RNA in the air, particularly near COVID-19 patients. In the medicine ward, the virus was detected at both 1- and 3-m distances, with a higher viral load (lower Ct values) at 1 m, indicating greater contamination closer to the patients. Similar patterns were observed in the ICU, where SARS-CoV-2 was found near ventilated patients. Despite the ICU's aerosol-generating procedures, viral concentrations were comparable to those in the medicine ward, likely due to similar contamination levels across both settings. In the emergency ward, even brief patient occupancy resulted in viral RNA detection in the air, with viral loads comparable to those measured at 3-m distances in other wards. **Figure 1** illustrates the decrease in viral load as distance from the patient increases, highlighting the risk of close-contact airborne transmission.

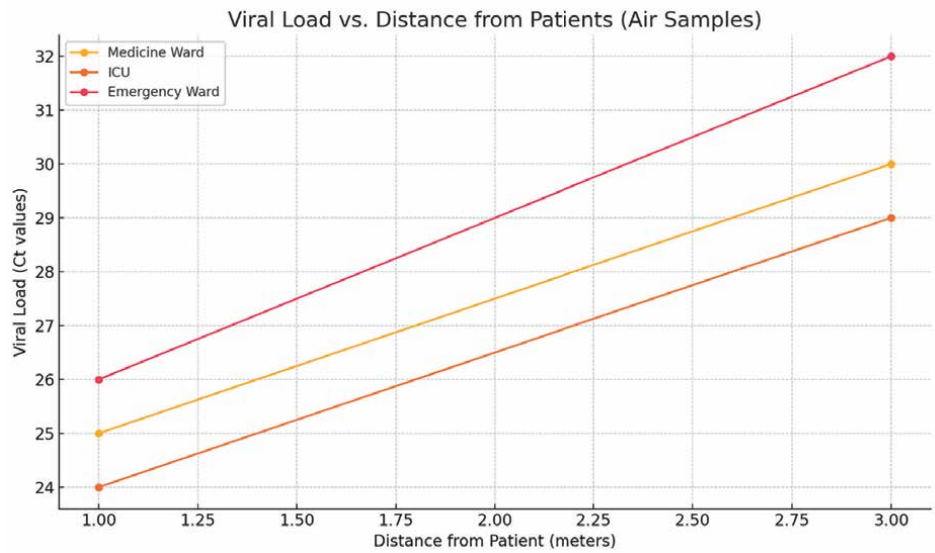

Figure 1.
Viral load vs. distance from patients (air samples).

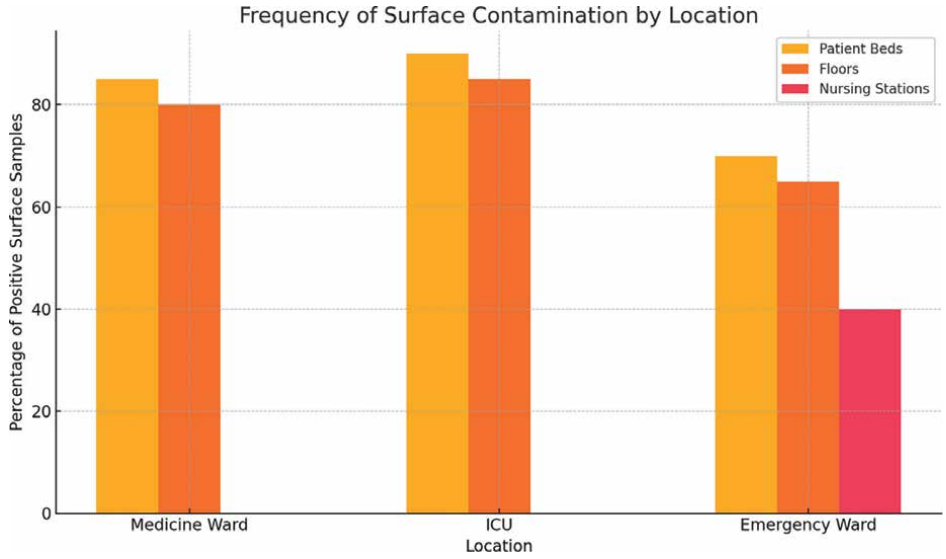

Figure 2.
Frequency of surface contamination by location.

Surface contamination was widespread in high-contact areas, with SARS-CoV-2 RNA detected on patient beds and floors in both the medicine ward and ICU, confirming these surfaces as potential sources of fomite transmission. Interestingly, nursing stations in these wards, which were separated by physical barriers, showed no contamination. In contrast, the emergency ward, lacking such barriers, exhibited positive surface samples, suggesting higher contamination risks in open-plan areas. **Figure 2** shows higher contamination rates on patient beds and floors across all wards, with notable contamination at the nursing stations in the emergency ward.

These findings emphasize a clear correlation between viral load and proximity to infected patients, with samples taken within 1 m showing consistently higher viral loads. Although the ICU had aerosol-generating procedures, its viral concentration remained similar to the medicine ward, likely due to lower patient density. The emergency ward displayed contamination levels comparable to other wards, despite the transient nature of patient stays.

Overall, the results underscore the influence of patient proximity, ward layout, and ventilation on viral transmission, highlighting the need for robust infection control measures to mitigate airborne and surface transmission in healthcare settings.

7. Discussion

The present study provides crucial insights into the airborne and surface transmission of SARS-CoV-2 in a dedicated COVID-19 hospital during the peak of the pandemic. By systematically assessing viral contamination in the air and on high-contact surfaces, our findings contribute to the growing body of evidence regarding the transmission dynamics of SARS-CoV-2 in healthcare settings. The detection of viral RNA in air samples up to 3 m from patients, coupled with extensive surface contamination, underscores the significant role of both aerosolized and fomite-based transmission routes in the nosocomial spread of COVID-19.

One of the key findings of our study is the higher concentration of viral RNA in air samples collected at a 1-m distance compared to those collected at 3 m. This observation aligns with previous studies that have demonstrated the presence of SARS-CoV-2 in aerosols near infected individuals, suggesting that viral load diminishes with increased distance from the source. However, the detection of viral RNA even at a 3-m distance reinforces the potential for airborne transmission beyond the traditionally recognized close-contact range of 1–2 m. This has important implications for infection control practices, emphasizing the need for adequate ventilation and air filtration systems in hospital settings to mitigate the spread of the virus [25].

Surface contamination was widespread across different areas of the hospital, with the highest viral loads detected on patient beds and floors. These findings are consistent with previous studies that have reported significant environmental contamination in healthcare settings, particularly in areas where COVID-19 patients are treated [29, 30]. The high viral presence on patient beds is expected, given their direct and prolonged contact with infected individuals. Contamination of floors, on the other hand, suggests possible viral deposition from respiratory droplets and aerosols, which subsequently settle on surfaces. Healthcare workers and hospital staff may inadvertently contribute to further transmission by coming into contact with these contaminated surfaces and unknowingly transferring viral particles to other locations within the hospital.

The presence of SARS-CoV-2 RNA on nursing stations and other high-touch surfaces such as door handles and medical equipment highlights the potential role of fomites in viral spread [26, 27]. Despite rigorous cleaning and disinfection protocols in place, our study suggests that there may be gaps in current sanitation practices or that rapid recontamination occurs due to frequent human interaction with these surfaces. This calls for enhanced cleaning protocols with increased frequency, as well as the use of more effective disinfectants to ensure complete viral inactivation. Additionally, the findings underscore the importance of consistent hand hygiene among healthcare workers and patients to minimize the risk of fomite-mediated transmission [11, 13, 31].

An important consideration in the interpretation of our results is the distinction between the detection of viral RNA and the presence of infectious, replication-competent virus. RT-PCR, while highly sensitive, identifies genetic material and does not necessarily indicate the presence of viable virus capable of causing infection. Nonetheless, the detection of viral RNA on surfaces and in air samples suggests that the potential for transmission exists and should not be underestimated. Further studies using viral culture techniques would be necessary to confirm the infectivity of detected viral particles and better understand the risk posed by environmental contamination [32–34].

Our study also highlights differences in viral contamination between different hospital areas, with the highest levels observed in the ICU, followed by the emergency ward and medicine ward. This gradient likely reflects differences in patient acuity, disease severity, and the nature of medical interventions performed in each setting. ICU patients, particularly those receiving high-flow oxygen therapy or undergoing procedures such as intubation, generate higher levels of aerosols, which may contribute to increased airborne viral loads. In contrast, the medicine ward and emergency ward, while still exhibiting significant contamination, may have lower viral concentrations due to variations in patient care activities and ventilation conditions.

The findings of this study reinforce the need for comprehensive infection prevention and control measures tailored to the specific risks associated with airborne and

surface transmission. Enhanced ventilation strategies, including the use of HEPA filtration systems and ultraviolet germicidal irradiation (UVGI), should be considered to reduce airborne viral loads in high-risk hospital areas. Additionally, the widespread use of personal protective equipment (PPE), including N95 respirators, face shields, and gloves, remains essential for healthcare workers to minimize the risk of exposure.

Our results also support the continued importance of physical distancing measures within hospital settings. While the detection of viral RNA at a 3-m distance suggests potential for transmission beyond conventional close-contact distances, the highest viral concentrations were still observed closer to patients. This indicates that while maintaining a safe distance remains a critical preventive measure, it should be complemented by other interventions such as mask-wearing and environmental controls to effectively reduce transmission risk.

Limitations of the study should be acknowledged. First, as mentioned earlier, RT-PCR detects viral RNA but does not confirm the presence of infectious virus. Second, variations in environmental factors such as airflow, temperature, and humidity were not systematically assessed in this study, though they are known to influence viral stability and transmission. Future research incorporating viral culture methods and detailed environmental analyses would provide a more comprehensive understanding of transmission dynamics in healthcare settings.

Despite these limitations, our findings have important public health and clinical implications. They reinforce the necessity for stringent infection control measures, not only within hospital environments but also in community settings where similar transmission mechanisms may be at play. The study also highlights the need for continuous monitoring of environmental contamination in healthcare facilities, particularly during outbreaks, to inform evidence-based mitigation strategies [15, 35].

This study provides valuable evidence supporting both airborne and fomite-based transmission of SARS-CoV-2 in a hospital setting. The detection of viral RNA in air samples at distances up to 3 m, coupled with significant surface contamination, highlights the critical need for robust infection control strategies, including enhanced ventilation, rigorous disinfection practices, and adherence to PPE protocols. As the global healthcare community continues to combat COVID-19 and prepare for future pandemics, understanding the environmental persistence and transmission dynamics of SARS-CoV-2 remains essential for optimizing disease prevention and control efforts.

8. Infection control implications

The study underscores the importance of implementing comprehensive infection control measures in healthcare settings treating COVID-19 patients. Improving air ventilation in hospital wards, particularly in high-risk areas like ICUs, emergency rooms, and medicine wards, is essential. Mechanical systems with HEPA filters are recommended, as natural ventilation alone may not provide sufficient air circulation in densely populated hospital spaces. Regular air monitoring should be adopted, allowing hospitals to detect viral contamination in real time and take immediate action if needed [36, 37].

Physical barriers, such as glass partitions between healthcare workers' stations and patient areas, have proven effective in reducing surface contamination and transmission risks. Healthcare facilities should install such barriers in high-risk zones. Additionally, high-touch surfaces, especially in wards where COVID-19 patients are treated, must be disinfected frequently. Strict cleaning protocols, using effective

cleaning agents against SARS-CoV-2, are crucial for maintaining hygiene and minimizing the risk of surface transmission [29, 30, 38].

The importance of air filtration systems, personal protective equipment (PPE), and cleaning protocols is evident in this study. Advanced air filtration, including HEPA filters and ultraviolet germicidal irradiation (UVGI), can help reduce airborne viral particles, particularly in areas where aerosol-generating procedures occur. PPE, including N95 respirators, face shields, gowns, and gloves, remains vital for healthcare workers, especially in areas with high airborne or surface contamination. Ensuring that healthcare workers consistently use appropriate PPE is critical in preventing both airborne and surface transmission [39, 40].

Cleaning protocols play a key role in preventing fomite transmission. High-touch surfaces must be disinfected regularly, with a focus on areas where COVID-19 patients are treated. Healthcare workers should be trained on proper cleaning techniques, and hospitals should enforce stringent cleaning schedules to reduce the risk of transmission [41, 42].

The findings also have broader policy implications for managing infectious outbreaks in hospital settings. Hospitals should adhere to enhanced ventilation standards, ensuring that all wards, especially those treating infectious patients, are equipped with adequate air filtration systems. Additionally, policies enforcing the mandatory use of PPE should be strictly followed, and healthcare facilities must maintain sufficient PPE supplies to avoid shortages during outbreaks [15, 35, 43].

Infection control policies should incorporate airborne transmission precautions, mandating the use of N95 respirators in high-risk areas. Regular surface disinfection protocols must be enforced, especially in high-traffic areas such as emergency wards. Hospitals should also implement real-time surveillance systems for air and surface monitoring to detect contamination early and adjust control measures as needed [44–46].

The safety of healthcare workers is paramount, and continuous training in infection prevention, proper PPE use, and hygiene practices is crucial. Hospitals should ensure regular health monitoring and provide psychological support to healthcare workers during outbreaks [47, 48].

The study highlights the importance of a multilayered approach to infection prevention and control in healthcare settings. By improving ventilation, ensuring consistent PPE use, and maintaining strict cleaning protocols, hospitals can reduce the risks of both airborne and surface transmission of SARS-CoV-2 [49, 50]. These findings provide valuable insights for hospital-level practices and broader health policy aimed at protecting healthcare workers and patients during future infectious outbreaks.

9. Limitations of the study

The study had several limitations. A key limitation was the use of RT-PCR, which detects viral RNA but cannot distinguish between live, infectious virus and noninfectious fragments. Without viral culture methods, it is unclear whether the detected RNA was capable of causing new infections. Additionally, the relatively small sample size and the focus on specific hospital areas (medicine ward, ICU, and emergency ward) limit the ability to generalize the findings to the entire hospital or other settings. Variability in patient viral load and contamination over time was not accounted for, and the study was conducted over a short period, coinciding with the pandemic's peak in Delhi, which may have affected contamination levels [47, 48].

The study also did not examine how contamination might vary during different times of day, such as during shifts or patient turnover, suggesting that longer-term studies are needed to capture these variations.

Future research should address these gaps by including viral culture techniques to assess the infectiousness of detected viral particles and by expanding sampling to a broader range of hospital environments. Longitudinal studies are needed to explore contamination patterns over time, and research on the effectiveness of different ventilation systems and cleaning protocols could provide deeper insights. Additionally, studies on the viability of the virus on different surfaces and intervention strategies, such as air purifiers and UV germicidal irradiation, are recommended to better manage airborne and surface transmission in healthcare settings.

10. Conclusion

This study provided key evidence of SARS-CoV-2 presence in both the air and on surfaces within a COVID-19 hospital in Delhi, highlighting the significance of airborne transmission, especially up to 3 m from patients. Surface contamination near patient beds and floors suggests that fomite transmission is also a risk. These findings stress the need for comprehensive infection control strategies, including improved ventilation, the use of N95 respirators, and regular disinfection of high-contact surfaces.

The broader implications for hospital infection control practices include enhanced airborne precautions, especially in high-risk areas like the ICU, where HEPA filters and robust ventilation systems are necessary. Strict hygiene practices and frequent surface cleaning must be enforced to mitigate transmission risks.

Further research should focus on determining whether detected viral RNA represents live, infectious virus, and expand sampling across various healthcare settings. Future studies should also investigate the effectiveness of different ventilation systems and infection control interventions, helping to refine measures to prevent the spread of SARS-CoV-2 and other pathogens in hospitals.

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Chapter 4

Perspective Chapter: Introduction to Mpox

Khayry Al-Shami and Manar Al-Shami

Abstract

The mpox epidemic (formerly known as monkeypox) became a major worldwide health issue after the COVID-19 pandemic. A new outbreak of mpox was discovered in the UK during May 2022, which rapidly expanded throughout Europe and the Americas and Africa, while the Americas reported most cases. Protective public health messages became essential when the virus crossed previously identified epidemiological chains. The orthopoxvirus-caused mpox infection presents mild symptoms like smallpox, except it affects unvaccinated individuals who develop more severe conditions. Individuals or animals who transmit the disease to others do so through direct contact, while the election of the symptoms features flu-like characteristics alongside specific rash development and lymph node inflammation. The termination of smallpox vaccination programs after the disease elimination in the 1980s resulted in mpox outbreaks among unvaccinated communities. The MVA-BN type of smallpox vaccine gives protection against various diseases, yet global mpox outbreaks persist without identifiable transmission pathways in affected populations. PCR assays and emerging T-cell-based tests play essential roles in distinguishing mpox from both smallpox and chickenpox infections. Severe mpox infections in young children and people with impaired immune systems might need antiviral treatment, but the effectiveness stays uncertain. The epidemiology, along with mpox transmission routes, clinical manifestations, and medical interventions, forms the core content of this chapter. The current situation demands worldwide disease surveillance combined with public health awareness programs and prepared emergency response capabilities to address future disease outbreaks, especially following the COVID-19 pandemic.

Keywords: Mpox, zoonotic disease, cross-protective immunity, global health preparedness, orthopoxvirus, Covid 19, vaccine

1. Introduction

The world has been working to slow down the SARS-CoV-2 virus's rapid spread and create a vaccine against the virus and its mutations ever since the COVID-19 pandemic began at the end of 2019. Early in 2022, as the world was beginning to recover from the COVID-19 pandemic, consideration turned to the errors that had

not included the SARS-CoV-2 virus in the global health system. This was because infectious disease threats persisted because of global population mobility [1, 2]. However, as seen by the epidemic of human smallpox cases that has spread over Europe, the Americas, Australia, and a portion of Africa since May 7, 2022, the persistent demands for the prevention of these contagious illnesses and their repeated warnings have gone unanswered [3, 4]. In May 2022, Health Security in the United Kingdom confirmed the first human case of mpox during the ongoing outbreak. The patient's travel history included visits to Nigeria, Africa. Two further instances were found to be residing in the same location a week later on May 14, 2022, although they had no prior history of travel inside or outside of the United Kingdom. At the same time, it was confirmed that they were not communicating with the case identified on May 7, which went to Africa [5]. Gradually, 12 WHO member countries from three different regions continued to confirm new outbreaks of mpox. About 28 suspected cases and 92 laboratory-confirmed cases of mpox were known as of May 21, 2022. Cases from the United States, Canada, Portugal, France, Spain, Germany, Sweden, Belgium, Australia, and the United Kingdom were reported to the WHO. An increase in instances is generally anticipated. Finding the currently unknown epidemiological connections between these instances is a pressing priority. Even though most medical professionals are unaware of mpox, particularly front-line healthcare workers in acute care/STI clinics, hospitals, and emergency departments, there is an urgent need for quick access to accurate, fact-based information that is clear and succinct [6]. As of right now, no fatalities have been documented; yet, some odd and unsettling elements of these epidemics require further investigation [4]. According to the weekly data covering February 13–19, there were 55.1% fewer new cases globally than the previous week (111 cases, February 6–12). Across the whole, the Americas accounted for 86% of cases and Africa for 6.4%. The top 10 countries worldwide affected, accounting for 84.9% of all cases reported worldwide, were Brazil (n = 10,808), the United States of America (n = 29,987), Mexico (n = 3828), Peru (n = 3752), Spain (n = 7538), Colombia (n = 4080), Germany (n = 3692), France (n = 4128), the United Kingdom (n = 3735), and Canada (n = 1460) [7]. This chapter covers the origin and development, the physiology, the spread, probabilities, history, and symptomatology of the monkeypox virus (MPV). Thus, in addition to identifying what is now known about therapeutics available for COVID-19, there is the era of this viral illness and vaccinating against it.

2. The origin of the mpox virus (history and epidemiology)

MPV, an orthopoxvirus belonging to the genus orthopoxvirus and family Poxviridae, was identified as a virus in sick monkeys in a Danish laboratory in 1958, about 20 years before it became a disease that affected humans [8]. During a time of plummeting smallpox incidence in the 1970s in the Democratic Republic of Congo (DRC), the first human mpox patient was seen in a nine-month-old boy brought in to the hospital. The MPV-like virus was isolated from a smallpox-like disease in the boy [7]. Six MPV cases were documented in Nigeria, Liberia, and Sierra Leone between October 1970 and May 1971. About 10 further cases were reported between 1971 and 1978, with the first occurring in Nigeria in 1971. Although some terrain types might encourage the development of viruses, mpox has affected thousands of people and spread to 15 nations globally, including 11 in Africa. Since mpox first appeared in Western and Central Africa in 1970, especially

in the Democratic Republic of the Congo, the majority cases documented have come from these regions [9]. In total, 47 reported cases with MPV were identified from 1970 to 1979; all occurred in Western and Central Africa [10]. They knew these areas to be home to large rainforests encircled by small villages below the population of 1000 inhabitants at most. Daily lives and cultures of the residents of these rural areas included wild hunting and being exposed to different wild species [11]. With such a long history of adaptation to the human host, we might think the causative agent of smallpox to be the variola virus. Some records [12] have it that vaccines against smallpox have been practiced in China since more than a thousand years ago. Jenner's 1796 cowpox vaccine marked the beginning of rational vaccine production since it uses a virus that is spread by host animals and may infect people with a very minor illness. The smallpox eradication journey officially started in 1980 [7]. It is thought that before the invention of the smallpox vaccination, the disease of moderate and intermittent severity due to orthopoxviruses, including the mpox virus, was occurring in some degree in the human population [13]. The viruses are carried in host animals and periodically reappear in human populations. The variola virus is somewhat less common and less genetically distinct than the smallpox virus [14], and the death rate from smallpox was of the order of 30–50% of the proportion of the people that became infected, while the rate of variola was about less than one percent. Smallpox has been eradicated through vaccination and/or preventive exposure to smallpox, and the number of human orthopoxviruses infections can also be reduced [15]. The mpox virus exhibits two separate species: the Congo Basin and West Africa, much like the smallpox virus, which split into two groups throughout time. According to reports, mpox mortality can reach 10% in the Congo Basin region, 1% in West Africa, and significantly higher among HIV patients [16]. The most significant human mpox virus (MPV) epidemic before the COVID-19 period happened in the United States in 2003 when an infected Gambian giant pouched rat was transported to Texas from Ghana together with 800 other animals. These animals unfold the virus to close by prairie puppies that had been then dispatched elsewhere [17]. The West African virus related to the outbreak within the US differed from the authentic African. Everything within the US was related to the inflamed animals, making tracing techniques and detections easier. In addition, there has been no demonstrated proof of human-to-human transmission. The incubation duration becomes barely exclusive from what was known, at a median incubation duration of 14.5 days [18]. There had been no pronounced deaths and no gender preferences. In addition, the American model differs in the morphology and quantity of pores and skin lesions, a more haphazard course [17]. There was an epidemic in Sudan in 2005, and this is now no longer in Western or Central Africa. The particular purpose for the advent is not always nicely understood; however, it is considered that it is because of the spread of contamination from neighboring endemic areas or migrating inflamed animals [8]. Characteristically, the Sudan outbreak did no longer display symptoms and signs distinctive to that of the West and important African outbreaks, nor did it display case fatality costs comparable to the United States outbreak [19], despite the fact that a Nigerian outbreak in 2017 yielded many showed instances of MPV [20]. Three of those who were uncovered to the virus delivered the contamination from Africa to the United Kingdom earlier than spreading it further. Among them, four instances had been related to touch with one of the patients, with one healthcare employee affected. It became the second outbreak outdoors of Africa after it unfolded inside the US [21].

2.1 The international spread of the human mpox virus before the COVID-19 era

The greatest outbreak of MPV occurred in the US in 2003, when an infected Gambian giant pouched rat was shipped to Texas along with 800 smaller animals from Ghana, and they transmitted the virus to nearby prairie dogs, which were sent elsewhere [17]. The West African virus associated with the outbreak in the US differed from the original African. Everything in the US had been linked to the infected animals, making tracing strategies and detections easier. In addition, there was no proven evidence of human-to-human transmission. The incubation period was slightly different from what had been known, at an average incubation period of 14.5 days [18]. There were no reported deaths and no gender preferences. In addition, the American version differs in the morphology and number of skin lesions, a more haphazard course [17]. There was an outbreak in Sudan in 2005, and this was not in Western or Central Africa. The precise reason for the appearance is not well understood, but it is considered that it is due to the spread of infection from neighboring endemic areas or migrating infected animals [8]. Characteristically, the Sudan outbreak did not show signs and symptoms dissimilar to those in the west and central African outbreaks, nor did it showcase fatality rates akin to the US outbreak [19], although a Nigerian outbreak in 2017 yielded many confirmed cases of MPV [20]. After being exposed to the virus, three people transmitted the disease to the UK from Africa and then outside of it. Among them, four cases were linked to contact with one of the patients, with one healthcare worker affected. It was the second outbreak outside of Africa after it spread in the US [21].

2.2 The international spread of the human mpox virus after the COVID-19 era

A total of 42 member nations from five WHO regions—the Americas, Africa, Europe, the Eastern Mediterranean, and the Western Pacific—have reported mpox cases to WHO since January 1, 2022. Furthermore, as of June 15, 2022, 2103 laboratory cases and one probable case that nonetheless caused one fatality were reported to the WHO. The mpox outbreak nonetheless impacts guys who have had intercourse with different guys, together with guys who have had intercourse with guys who have lately mentioned having intercourse with new or a couple of partners. Epidemiological investigations are ongoing, and all through the current outbreak, maximum instances had been mentioned via sexual fitness or different fitness offerings in primary or secondary care centers wherein the character has a record of journey wherein the people journey has been predominantly to Europe, North America, or other international locations, now no longer formerly recognized to be related to this virus and increasingly in current local or no journey at all. An outbreak is one united state of America with showed instances of mpox. Mpox unexpectedly regarded in a couple of locations without an apparent epidemiologic connection to in advance locations, which indicates long-time undetected transmission. This is the primary time in quite a few specific WHO areas that quite a few mpox instances and clusters had been mentioned at the identical time. However, mortality has endured to be low for this cutting-edge outbreak, and WHO regards the hazard for the worldwide as moderate [22].

3. Overview of Monkeypox

3.1 The Orthopoxvirus: Mpox and related viruses

Monkeypox virus (MPXV) belongs to the Orthopoxvirus group (look at **Figure 1**) within the Poxvirus family that causes disease in animals and humans. It has a dsDNA of about 197 kb, including approximately 223 ORFs, and has variola virus (VARV, which causes smallpox and has a human host), vaccinia (VACV), camelpox (CMPV), and cowpox (CPXV), which affects both humans and animals [23, 24]. These illnesses are caused by immunologically related and mutually protective orthopoxviruses [25]. The three parts of the MPXV genome are the core region, the left arm, and the right arm, which are inverse terminal repeats. The arms, which are less conserved, are important in virulence and host specificity, whereas the core region, which is well maintained, is devoted to gene synthesis, replication, virion assembly, and the synthesis of around 181 proteins [26]. Recent studies have also shown that single-nucleic-acid polymorphic sites, multicopy genes, repeats, and recombination fragments are mainly located in the variable region, as is evidenced by the full-length MPXV genomic sequence information available to date [27]. Sequence alignments were in line with MPXV being a species in the genus Orthopoxvirus but not a direct derivative or progenitor of VARV, the agent of smallpox [28]. When the amino acid sequences of the monkeypox virus strain Zaire-96 and two variola virus strains, India-1967 and BSH-75, were compared, it was discovered that the nucleotide sequences encoding structural proteins and essential enzymes in the core region of the MPXV genome were 96.3% identical to those of VARV, while the terminal regions, which contain the majority of virulence and host-range genes, showed 83.5% to 93.6% identity with VARV [29].

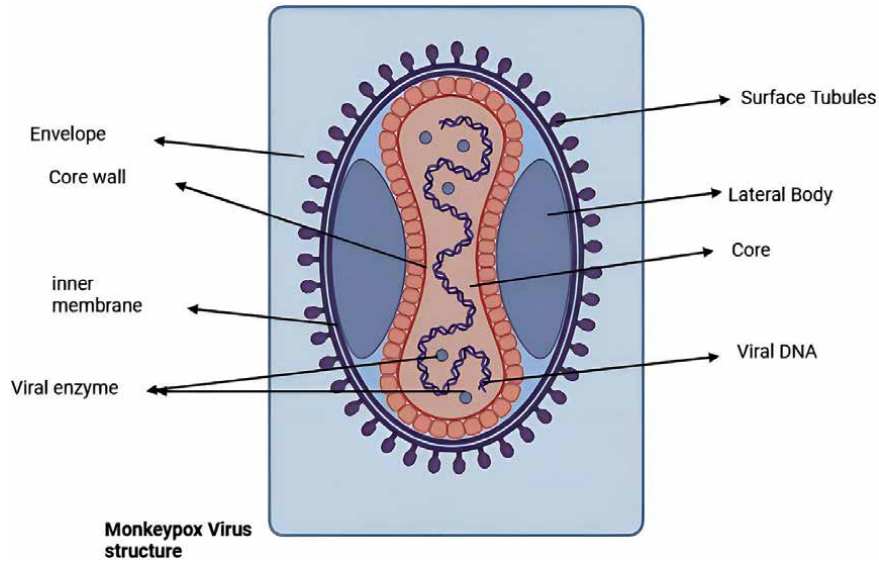

Figure 1.
Morphology of Monkeypox virus.

Two additional genes present in MPXV but absent or fragmented in VARV include COP-B7R, an endoplasmic reticulum (ER) resident protein potentially involved in apoptosis or to retain a secreted or cell surface protein in the ER important for immune response (e.g., secreted lipoprotein), and BR203, COPE7R, COPK4L, COPB12R, and COPK1L [30]. In contrast, genes in VARV but absent or fragmented in MPXV include COP-C10L, an IL1β binding protein that enhances virulence by blocking IL1 receptors and allowing the virus to escape IL1 activities; COP-E3L, an IFN-resistant protein with a C-terminal RNA-binding domain and an N-terminal Z-DNA-binding domain affecting the kinetics of cytokine IFN's anti-viral activities; and COP-K3L, another IFN-resistant protein affecting virulence through In addition, proteins of MPXV but fragmented in VACV include COP-B19R, an IFN-α/β binding protein, preventing IFN-α/β binding to its receptors and concomitantly preventing specific pathways from signaling [31]. ECTV virulence depends on these proteins, including BR-05/BR-226, a TNF-binding protein that is a TNFα and TNFβ binder, and BR-207, a serpine-2/apoptosis protein [32]. Potential objectives for diagnostics and for destiny subunit vaccines in opposition to mpox, which can be expressed in MPXV, however lacking in VACV, encompass proteins [33]. Each of the 6379 bp terminal inverted repetitions (TIR) in the MPXV genome has a putative telomere decision sequence, short tandem repeats, and a section of period 24.6 that shows the consensus oligomer sample for the crucial information of the recombinational sites, R1F4. These TIRs are similarly oriented but opposite. Using analysis aided by a laptop, 190 open analyzing frames containing >/= 60 amino acid residues were identified. Among those, four emerged inside the inverted terminal repetition. MPXV contains essential orthopoxvirus genes along with a collection of genes purportedly immunomodulatory and host-range. The OPG191 gene, which encodes the MPXV gp168 protein, and a coding location that changed into diagnosed on the 3′ terminus had been constant in a latest frameshift mutation primarily based totally on a 2-base insertion. With this insertion, the virus populace that changed into commonplace in 2022 changed into in advance truncated a few of the protein [34]. In the monkeypox virus, transmission—the pathogenicity and pathophysiology—starts with flip to transmission, observed with near touch of animal to human or human to human. Monkeypox and smallpox infect oropharyngeal (throat) or respiratory (nostril and mouth) mucosa of a host. The virus enters the inoculation webpage and replicates in respiration and oropharyngeal mucosa. Primary viremia outcomes in viruses spreading to the nearby lymph nodes. But those viruses attain lymph nodes and organs through blood movement in secondary viremia. This procedure represents the incubation period of seven to 14 or 21 days [35]. The maximal virion attachment in monkeypox likely involves extracellular matrix components, mobile glycosaminoglycans at the surface of the target cell, and outer virion proteins. Poxviruses are thought to enter host cells by either direct fusion with the plasma membrane at an unbiased pH or low pH endosomal routes, which release the viral core into the cytoplasm. For intracellular mature virions and enveloped extracellular virions to fuse with the cell, a complex of 12 non-glycosylated viral membrane proteins is required [36].Once the virus is encrypted, multi-subunit DNA-dependent RNA polymerase is brought into the virus, and the virus transcription is initiated, including the translation of early intermediates and late viral proteins from host ribosomes [37]. Inside these 'factories,' DNA synthesis takes place in cytoplasmic structures that develop from compact DNA wrapped in endoplasmic reticulum membranes into later crescent-shaped structures in which virions are assembled. With few exceptions, most mature virions persist within the cell (intracellular mature virions), while others are transported to the cell surface and enveloped by two membranes from the endoplasmic

reticulum or Golgi. Depending on their release out of the cell, the enveloped virions can either trigger actin polymerization (on which the particle is propelled by an actin tail onto an adjacent cell) or exit the cell after fusion of the cytoplasmic membrane and become round, enveloped, extracellular virions (**Figure 2**) [38].

3.2 Key symptoms and clinical presentation

Historical signs of mpox infection include recent travel to endemic areas, contact with wild animals introduced to endemic areas, and caring for an infected animal or person. But what matters most are symptoms. After infection, the mpox virus spreads by either of the following routes: intradermal, nasopharyngeal, or oropharyngeal. It then makes its way to nearby lymph nodes. Viral proliferation and subsequent seeding to different organs are caused by initial viremia. This illness takes seven to 14 days to incubate, with a maximum of 21 days (**Figure 3**). Lesions develop 1–2 days after secondary viremia and prodromal signs, including fever and lymphadenopathy. Patients that are infected may be infectious right now. Therefore, the initial lesions start in the oropharynx and spread to the skin, at which time serum antibodies are detected in serum [3]. In addition to fever, headache, myalgia, weariness, and lymphadenopathy, mpox differs from smallpox by presenting first with mpox. 1 to 2 days later, the mucosal lesions develop in the mouth, and the skin lesions become centrifugally concentrated on the face and extremities (palms and soles). The rash spreads to other parts of the body, or does not, and there can be a few or many thousands of lesions [39].

Lymphadenopathy in the inguinal, axillary, or cervical areas occurs, commonly before or after rash [40]. The lesions go through malar, papular, vesicular, and pustular phases during a period of 2 to 4 weeks, each lasting 1 to 2 days. These are deep-seated, hard lesions that range in size from 2 to 10 mm and alter simultaneously. Lesions are in the pustular phase for 5 to 7 days prior to the formation of crusts.The illness usually resolves 3–4 weeks after the onset of symptoms; however, the crusts build and desquamate during the next 7–14 days. During the next 2 to 4 weeks, the lesions progress through the macular, papular, vesicular, and pustular phases in 1- to

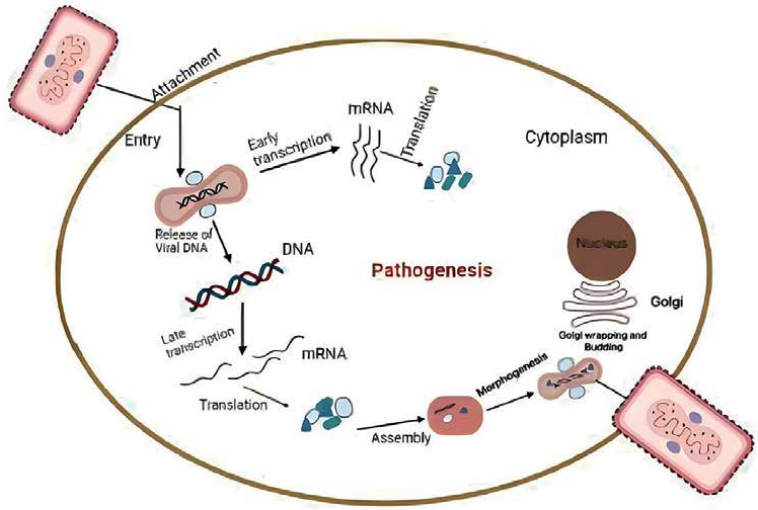

Figure 2.
Pathogenesis of monkeypox virus.

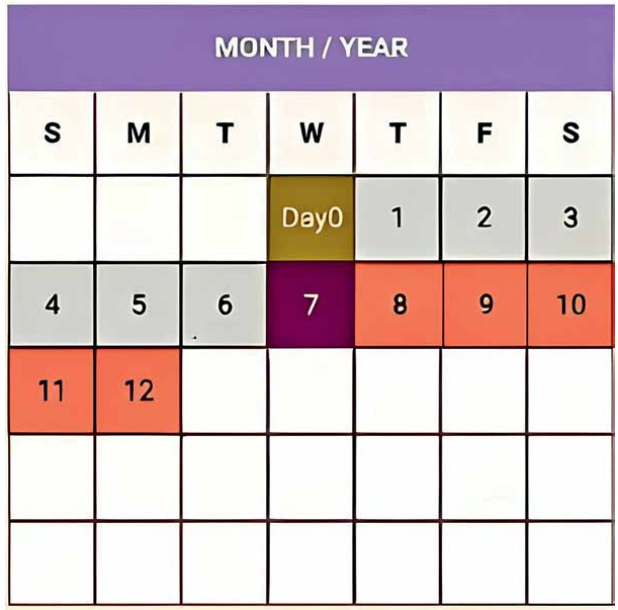

Figure 3.
Monkeypox infection timeline providing insight into the pathophysiology throughout the initial 12 days of infection. Around the second day, the first replication takes place in the main inoculation site. The first signs of monkeypox (secondary viremia) appeared after day 7 of infection, and the disease spread to other tissues. This involves a feverish sickness that lasts 2 to 3 days and often appears 10 to 14 days after the original exposure. Additionally, the distinctive skin lesions appeared.

2-day increments. They are solid, deep-seated lesions that are 2–10 mm in size. At the same time, they shift. Before crusts develop, lesions spend 5 to 7 days in the pustular phase. The illness usually resolves 3–4 weeks after the onset of symptoms, with crusts forming and desquamate during the next 7–14 days. **Figure 4** shows that the patient is no longer infectious once all of the crusts have disappeared.

The virus that causes varicella (chickenpox) infection (varicella zoster virus or VZV) looks a lot like herpes simplex virus (MPV), and the two are often confused, especially in places where MPV is common. But each infection is distinguishable by different characteristics of illness. For example, the onset of VZV patients is usually brief and mild febrile, with a rapidly growing (1–2 d) pleomorphic rash (characterized by neighboring lesions ranging from early to late) or no febrile prodrome. Over the course of the following two to four weeks, the skin lesions develop in one to two-day increments through the macular, papular, vesicular, and pustular stages. Lesions are firm, deep-seated, and 2–10 mm in size, and they alter synchronously. Before crusts develop, lesions stay in the pustular phase for 5 to 7 days. Over the course of the following 7 to 14 days, crusts develop and desquamate, and the disease usually resolves 3 to 4 weeks after the onset of symptoms. The patient is no longer regarded as infectious until all of the crusts have gone off [33].In areas where herpes simplex virus (MPV) is prevalent, varicella zoster virus (VZV) and MPV infections are frequently confused because of their similar appearances. But there are a number of traits that set one virus apart from another. For instance, patients with VZV often experience a brief, mild febrile prodrome, or none at all, followed by a pleomorphic rash that grows quickly (1–2 days) and may include surrounding lesions at different stages of development. In addition to being surface rather than underlying to the skin, VZV

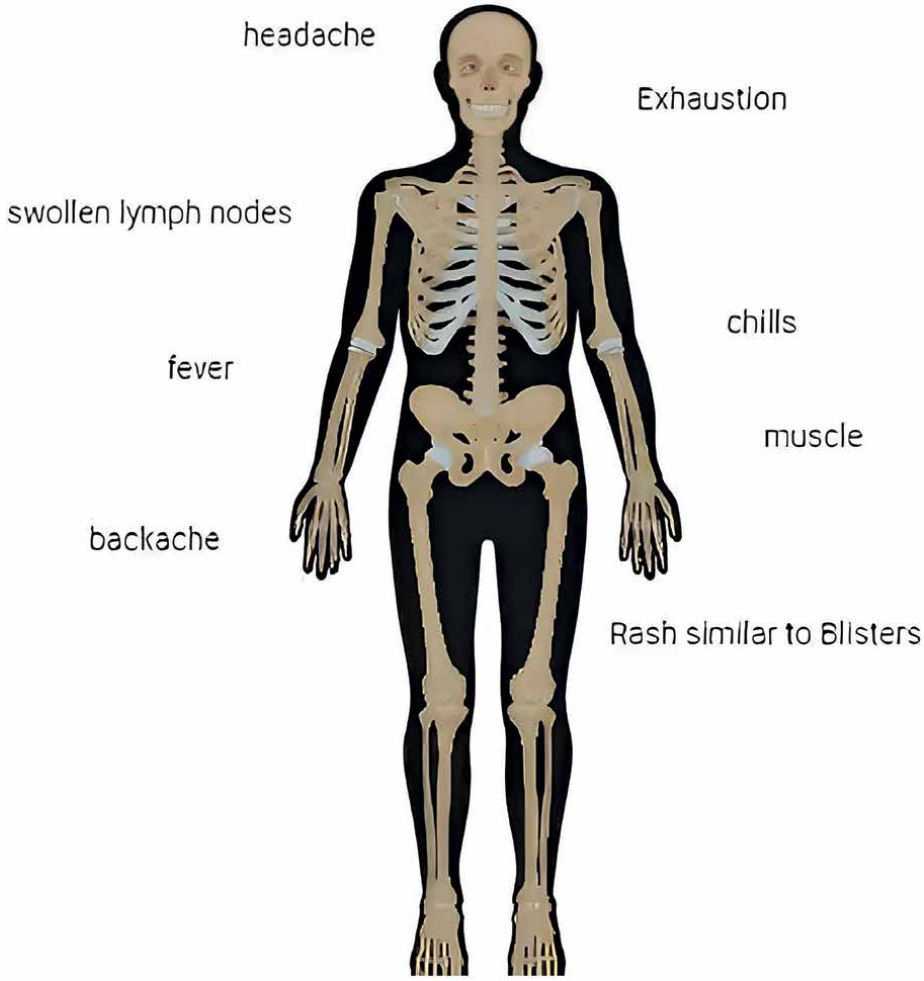

Figure 4.
Symptoms of monkeypox virus.

lesions differ from MPV lesions in that they have irregular boundaries. Additionally, the varicella lesions often spread in a centripetal direction from the body's perimeter. Although they are uncommon, VZV infection lesions have occasionally been observed in the palms of the hands and soles of the feet. Distinguishing smallpox and varicella from MPV is further aided by the rarity of discharge of late severe lymphadenopathy in VZV patients. Other herpetic illnesses (VZV), drug eruptions, syphilis, yaws, scabies, and rickettsial pox are among the conditions that might be mistakenly identified as MPV. This enhanced case detection and laboratory testing can also be completed in all suspected MPV endemic areas, leading to more accurate and speedy case discovery, better quality surveillance data, and better patient treatment, as well as a clinical case definition that can aid in differentiation of MPX from other conditions [40].

3.3 Transmission modes

MPV is spread two ways: from humans to animals or from humans to humans. Infection can be spread in humans if a skin lesion is present; infection is spread

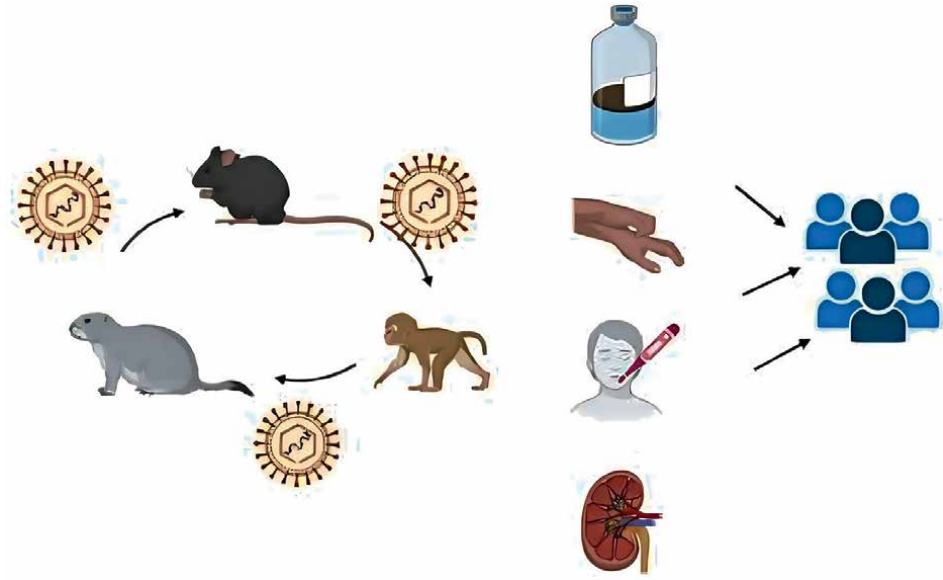

Figure 5.
The path by which MPV spreads and is transmitted. MPV may spread through two different channels: human-to-human and animal-to-human. Skin sores from an infected individual, contaminated patient environments or objects, respiratory droplets, and contact with body fluids have all been implicated in human-to-human transmission. In addition to inoculation through an infected animal's mucocutaneous sores, zoonotic transmission can also occur through direct contact with or consumption of one of the natural viral hosts.

through respiratory droplets, the contaminated environment of the patient, or through contact with body fluids (**Figure 5**). Compared to West Africa, the Congo Basin clade virus is more severe and potent, which is probably why it infects more people [41]. Zoonotic transmission is when the virus spreads from animals to humans in direct contact with any of the virus hosts, so this is how the virus is spread from animal to human. Contact with blood or fluids from an infected animal can also result in animal transmission [42]. The results of extensive investigation and continued tracking led them to discover that the family of the nine-month-old ate monkeys from time to time, indicating that monkeys make important contributions to the disease. The first isolation of the virus occurred in 1958 [43] from captive monkeys, with a fairly variable range of possible host animals, including rodents, squirrels, and bats [44]. In order to identify the definite reservoir, many studies were performed, but none concluded anything. Mpox outbreaks happened in rural villages and around rain forests; thus, the principal way to contract MPV was through direct contact with the sick animals, either through eating or hunting [45]. The virus may, of course, go from animal to human, but as of now, there is no evidence that it can spread from human to animal. The reproduction number (R0) of the Congo Basin clade is significantly larger than that of the West African clade due to the increased rates of human-to-human transmission, secondary attack rates (SARs), and serial transmission events. In addition to spreading quickly among people, the virus will continue to exist among these people [46].

As shown in many reports, the highest affected age group was less than 15 years old. Since then, the mpox vaccine has also given cross-protective immunity, or herd immunity, against mpox, as both were given against smallpox, and the affected age group has been increasing since the eradication and the ending of smallpox vaccination in the 1980s [47] since the eradication and the ending of smallpox vaccination

around the 1980s. It should come as no surprise that our vaccinated populations had fewer severe presentations and minor complications and lower rates of mortality [48]. A large majority of primary cases were adult men, who are more highly exposed to wild animals and hunting practices than women and children. Females and children, however, were the majority of secondary cases [49]. When secondary instances were mentioned, the main means of human-to-human transmission were big droplets released while coughing or sneezing, bodily fluids, or contaminated household items [50]. Although there have been reports of nosocomial viral infections, these are uncommon, and healthcare workers are at risk of catching the virus from extended patient contact [51]. Vertical transmission (across the placenta) and transfer of the virus by open wounds or scratches, using the same household items, and sleeping in the same room with a victim were also thought to make a person more likely to catch the disease [8]. Surprisingly, lesions were easily seen and found earlier in people with light skin due to their ease of detection compared to patients with relatively lighter skin color. A risk factor somehow affected mortality and morbidity [17].

4. Diagnosis

The Centers for Disease Control and Prevention (CDC) developed case-defining criteria for mpox as an infectious illness during the 2003 epidemic in the United States. Naturally, though, endemic environments will not benefit as much from the same standards. As the community is exposed to more diseased humans or mammals, the specificity of epidemiological criteria declines. Furthermore, when the incidence of similar conditions increases, clinical criteria become less specific. This is the situation with chickenpox in Africa, where there is a lack of a systematic varicella zoster vaccination [52]. The main reservoirs of mpox animals have yet to be identified, but it is thought rodents such as Gambian giant rats and snake squirrels act as a reservoir [53]. It is thought that sources of this infection in humans come from close contact with infected animals or from cuts in the skin associated with handling or eating infected animals. In some cases of human mpox in the United States, respiratory transmission from an infected animal to a human had been found. In addition, human-to-human transmissions appear to be due, at least partly, to respiratory droplets [33]. The incubation period is 10–14 days after exposure and infection; the prodromal period is 2 days. But in areas where the disease is endemic, the same standards might not be as helpful. The specificity of epidemiological criteria decreases as the population's possible exposure to diseased humans or animals increases. Additionally, the specificity of clinical criteria declines as the incidence of similar conditions rises, as is the case with chickenpox in Africa because there is no standard varicella-zoster vaccination. Although the primary mpox animal reservoirs have not yet been determined, rodents like snake squirrels and Gambian giant rats are thought to be two of them. Close contact with infected animals and cuts in the skin from handling or eating infected animals are thought to be the sources of infection from animals to humans. In certain human mpox cases in the US, respiratory transmission from an infected animal to a human was found. In addition, respiratory droplets are believed to be involved in some human-to-human transmissions. There is an incubation period of 10–14 days after exposure and infection, followed by a prodromal period of 2 days. Infected persons may have a fever, chills, malaise, headache, and back pain, as well as sore throat, shortness of breath, and occurrence of lymphadenopathy (swollen lymph nodes). Most (about 90 percent) of all human mpox infections result in lymphadenopathy (enlargement of lymph nodes) in

the submandibular, neck, or groin regions. This trait distinguishes human MPV infection from smallpox infection.Following the prodrome phase, the mpox maculopapular rash can develop with a diameter of up to 1 cm. However, the same criteria may not be as beneficial in regions where the illness is prevalent. The specificity of epidemiological criteria decreases as the population's possible exposure to diseased humans or animals increases. Additionally, the specificity of clinical criteria declines as the incidence of similar conditions rises, as is the case with chickenpox in Africa because there is no standard varicella-zoster vaccination.Although the primary mpox animal reservoirs have not yet been determined, rodents like snake squirrels and Gambian giant rats are thought to be two of them. Human-animal transmission is believed to occur through close contact with diseased animals and skin injuries from handling or consuming infected animals. The submandibular, neck, or groin regions exhibit lymphadenopathy in over 90% of all human mpox infections. It is a defining property that can differentiate smallpox infection from human MPV infection [54]. The mpox maculopapular rash develops progressively following the prodrome stage and ranges in diameter from 0.2 to 1 cm. It is the time when the virus is most infectious and can spread to other people. Second, these skin lesions begin on the face and torso before spreading to the palms and soles of the arms and legs. Lesions start as macules, papules, vesicles, and pustules and progress through several, frequently distinct phases in a short period of time (2 to 4 weeks) (**Figure 3**). The scabbing and desquamation, which could be a part of the crusting phase in some cases, can cause dyspigmented scars [9, 33].

4.1 Laboratory diagnosis

The similarity in the clinical features of smallpox and mpox was the reason laboratory diagnosis was important. Reference labs can develop a wide array of laboratory tests for distinguishing these viruses, using the great similarity in the genomes with a high degree of separation in host immune responses to orthopoxviruses. Two genes targeted by the MPV can be diagnosed using molecular techniques such as real-time polymerase chain reaction (PCR) assay. Using one of the PCR assays, it can detect nearly 13 different Eurasian orthopoxviruses by targeting the DNA polymerase gene (Copenhagen (COP) E9L gene). MPV is specific and sensitive to MPV, as another assay is mounted against the single nucleotide polymorphisms found in the MPV (COP-B5R gene), which encodes for the virus' envelope protein [55].

The main disadvantage of these DNA tests is that they require the person being tested to be in an active part of infection while the virus is still present. It can not be used in mpox diagnosis when the infection clears. To circumvent that, scientists developed other techniques based on the host's immune response to the MPV. Yet, due to the difficulties associated with developing a standard antibody test for mpox, cross-reactive immune responses from previous smallpox vaccinations complicate the test. However, an immunoglobulin M (IgM) antibody test is utilized to identify MPV infection since prior smallpox immunization does not produce anti-vaccinia IgM antibodies [56]. The entire viral enzyme-linked immunosorbent test (ELISA), which quantifies the antibody titers to the mpox and vaccinia viruses and their ratio, is an additional strategy. ELISA was utilized to differentiate between current mpox infection and previous smallpox vaccination using a peptide expressed by the mpox homolog of the cowpox virus strain Brighton Red (BR) 219 gene. Cellular immunity responses that need an antibody response to certain infections, however, could be a novel and improved diagnostic target above the earlier tests. The assessment of multiple orthopoxvirus-specific T lymphocytes is one such method that is currently being developed [57].

5. Treatment and prevention of MPXV infection

5.1 Supportive treatment

Only supportive symptomatic treatment is required for the majority of MPXV infection patients, who recover without medication therapy [58]. It includes a wide range of active care techniques and pain control, making sure patients are getting enough food and water and protecting sensitive areas like the eyes and genitalia. In addition, it is necessary to prevent and treat secondary bacterial infections and other care-related problems [59]. Treatment options for severe MPXV-induced discomfort include oral laxatives for severe anal pain and pain medications such as acetaminophen, ibuprofen, lidocaine gel, and metamizole. Rectal suppositories that include steroids or emollients are additional effective topical analgesics. In extreme situations, opioids may be utilized. When the patient has gastrointestinal symptoms such as diarrhea and vomiting, it is necessary to replenish fluids and nutrients [60].

5.2 Antiviral drugs and intravenous (intravenous) treatment of vaccinia immune globulin (VIGIV)

Other medications for treating MPXV infections include cidofovir, brincidofovir, and tecovirimat. However, even with human dose trials, the effectiveness of these medications has not been completely determined. Tecovirimat (TPOXX, ST-246) is prescribed to treat human VARV infection in adults and children weighing more than 3 kg. It accomplishes this by blocking the overlap of the orthopoxvirus VP37 envelope-wrapping protein. Tecovirimat may be taken orally for a period of 14 days, either as capsules or intravenously [61]. The medications do not need hepatic/renal function adjustments when taken orally but are not given intravenously to patients with severe renal impairment. The most frequent adverse effects of oral administration include headache, nausea, stomach discomfort, and vomiting, while the most frequent adverse effect of intravenous treatment is an injection site response. Patients using repaglinide should exercise caution as it may raise their risk of hypoglycemia. Additionally, tecovirimat has not been demonstrated to be useful in treating VARV in people, and it could be less effective in individuals with impaired immune systems. In an animal investigation, tecovirimat-treated mice displayed greater clinical symptoms of illness than placebo-treated animals [62].

In June 2021, the United States Food and Drug Administration (FDA) approved brincidofovir (BCV) for the treatment of human VARV disease under the Animal Rule. Brincidofovir is a broad-spectrum, long-acting antiviral agent that is orally available and selectively targets orthopoxviruses by inhibiting viral DNA synthesis mediated by DNA polymerase. As an analog of the intravenous medication cidofovir, it may be less harmful to the kidneys than intravenous cidofovir [63]. In spite of this, the Centers for Disease Control and Prevention (CDC) and the literature advise treating MPXV disease with BCV. Increased bilirubin or hepatic transaminase levels, nausea, vomiting, diarrhea, and abdominal pain have all been associated with BCV treatment [64].

Although BCV is not nephrotoxic, it can increase liver transaminase levels; hence, liver function tests should be kept an eye on. Although the safety profile of using BCV to treat human VARV illness is excellent, research is still being done. It is not advised for women who are pregnant or nursing. VIGIV was licensed by the FDA to treat post-vaccination problems. However, it is not authorized to treat MPXV. The CDC

allows the use of stored VIGIV to treat orthopoxviruses, including MPXV, during an outbreak. For the treatment of patients with progressive vaccinia, eczema vaccinatum, severe generalized vaccinia, widespread body surface involvement, or periocular implantation due to unintentional inoculation, VIGIV offers two approved intravenous formulations (Dynport & Cangene). VARV or MPXV immunization after MPXV exposure is not advised for those with significant immunodeficiency in T-cell function, for whom VIGIV is required for prophylaxis. However, the effectiveness of VIGIV in preventing MPXV infection is still little understood. The use of VIGIV for MPXV has not been shown to be beneficial, and it is uncertain if using VIGIV to treat a severe MPXV infection will be beneficial. Healthcare professionals should, however, think about using it in situations of serious illness where the same strong antibody response may be compromised [65].

5.3 Monkeypox vaccines

After the disease was eradicated 35 years ago, smallpox vaccination stopped. Thus, today, a considerable part of the world population does not possess immunity to either smallpox or any other zoonotic orthopoxvirus infection. However, years or decades after receiving a smallpox vaccination, one may develop cross-protective antiviral immunity against other related viruses, such as MPXV. Currently, four smallpox vaccinations that have received emergency permission to prevent MPXV infections are in use. The vaccines in these groups are the Aventis Pasteur Smallpox Vaccine (APSV), a strong live vaccine, and two attenuated live vaccines, LC16m8 and the modified Ankara–Bavaria Nordic (MVA–BN) vaccine. The first-generation vaccine is not used anymore. APSV, also known as WetVax, is an animal reaction vaccine made using untreated vaccinia virus. It is applied to large animals, such as cattle, and the resulting scabs are then collected for extraction. The vaccine is a liquid formulation of a calf lymph origin (replication competency) vaccinia virus. Available under IND or EUA for use in the prevention of smallpox in circumstances where licensed vaccines are not available or contraindicated, ACAM2000 is a second-generation smallpox vaccine developed by Sanofi Pasteur Biologics. It is a live attenuated, replication-competent vaccinia strain, developed in the US for use in emergency situations for VARV before the 2022 MPXV outbreak [66]. Despite this, however, the vaccine it offers is fraught with a risk of side effects, which include myocarditis, pericarditis, ocular complications, and even blindness in some people. Therefore, active vaccination against smallpox sickness is recommended for those at high risk of contracting VARV, but not for children, newborns, pregnant women, or those with eczema or other skin conditions.

LC16m8 (Kaketsuken) is a third-generation smallpox vaccine that has a license in Japan. The Lister strain is the source of the vaccinia virus because it uses a live, highly attenuated, reproducing strain of the virus. The neurotoxicity of the vaccination has dramatically reduced since it was initially used in the 1970s [67]. It is also very immunogenic, and with a single dosage, it may produce strong humoral and cellular immunity as well as long-term immunological memory in animal models. However, it is yet unknown if this vaccination prevents human MPXV infection. However, since the 2022 MPXV epidemic in Japan, LC16m8 has been used for postexposure prophylaxis in certain Japanese locations [68]. The modified Vaccinia Ankara-Bavaria Nordic (MVA-BN) smallpox vaccine is a live viral vaccine that has been widely used worldwide, including in the UK, Europe, and the USA. It is derived from the MVA-BN strain, a highly attenuated, nonreplicating orthopoxvirus. The replication-defect variant of the MVA-BN smallpox vaccine is injecting modified vaccinia into chicken

embryo fibroblasts and repeatedly administering the vaccine until certain genes are eliminated, resulting in a vaccine that is immunogenic but does not replicate. The MVA-BN vaccine has one great advantage over the original smallpox vaccines, in that it cannot replicate if you get vaccinated with it. Additionally, it has demonstrated high immunogenicity and a good safety profile in more than a dozen clinical studies. Strong cellular activity and humoral immune response, and the ability of the MVA-BN vaccine to stimulate a response in people with pre-existing immunity to vaccinia, have been shown for it. Administration of two 0.5 mL doses of the vaccine subcutaneously and 4 weeks apart is optimal for generating immunity. High-risk people are protected against MPXV infection by MVA-BN homologous vaccines, which use a priming dose to prime the immune system and a second (booster) dose of the identical vaccination to prevent MPXV infection before and after exposure [69]. Heterologous vaccines are more complicated than the currently existing vaccines, which are also easier to create and license.

6. Conclusion

Mpox has now become one of the most pressing global health concerns now and more so after the COVID-19 pandemic. The fact that it spread beyond endemic areas shows that there are weaknesses in the world's health systems and that people still have to be ready for zoonotic diseases. Like smallpox, mpox presents clinically very similar signs; however, the fact that the diseases are less severe and deadly than smallpox has not reduced the drive of the necessary quick medical interventions. With smallpox vaccination stopped, populations are vulnerable, and epidemiological cross-protection is required, especially for those at high risk. Presently, there exist shortcomings in diagnosing and treating the disease even if there are diagnostic instruments and treatments for the sickness are available. As for the future, there is a crucial necessity in improving the international net-based monitoring, the work with the population, and education about preventive vaccines. Mpox tells us more about the means and ends that new diseases are ever a real factor for consideration, hence the need to be prepared for any move in the wake of disease outbreaks. Continuing to be hyperaware and funding investigations and precaution steps will be essential to keep mpox and other, possibly even more extensive, diseases from escalating into much larger conditions.

Notes/thanks/other declarations

This work is dedicated to Mr. Ali Al-Shami and Dr. Abeer Talafha.

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Chapter 5

Monkeypox Disease Epidemiology and Virus Ecology: From Neglected to High Consequence Infectious Disease

Bien-Aimé M. Mandja and Jean-Paul Gonzalez

Abstract

This chapter examines the epidemiology and ecology of the monkeypox virus (MPXV), focusing on its emergence, transmission, geographical spread, reservoirs, and hosts, while highlighting its growing impact on global public health. MPXV was first isolated in 1958 in monkeys at a Danish laboratory. The first human case appeared in 1970 in the Democratic Republic of Congo (DRC), coinciding with smallpox eradication efforts. The WHO supported active surveillance of MPXV from 1981 to 1986. While the natural reservoir of MPXV is still unknown, it is likely tree squirrels and wild rodents. Transmission occurs through animal-to-human (primary) or human-to-human (secondary) contact, involving close contact or inhalation of respiratory droplets. Sexual transmission was also reported among men who had sex with men (MSM) during the 2022 outbreak. Historically, MPXV cases were mostly limited to Central and West Africa. In 2005, cases emerged in Sudan, and in 2003, the first cases outside Africa were reported in the USA. The 2022 epidemic, with significant human-to-human transmission, led the WHO to declare an international public health emergency in July 2022. Between 2022 and 2023, nearly 100,000 cases were confirmed in 117 countries, with a case-fatality rate under 0.1%, mainly affecting MSM.

Keywords: epidemiology, risk factors, mpox emergence, mpox outbreaks, mpox pandemic

1. Introduction

The monkeypox virus (MPXV), today classified as *Orthopoxvirus monkeypox*, or monkeypox virus [1], is responsible for the mpox disease (formerly monkeypox disease) [2].

MPXV was first isolated in a Danish laboratory in 1958 from smallpox-like skin lesions on monkeys from Singapore, hence the name "monkeypox." Prior to this discovery, in India in 1936, a major smallpox-like epizootic was described in Bengal rhesus monkeys and could hypothetically be associated with the mpox virus [3]. In addition, other mpox epizootics were also observed in some non-human primates

(*Cynomolgus spp.*, *orangutans*, *gorillas, Cercopithecus spp.*, *marmosets*, *Macaca philippinensis*, *Macaca mulatta*, and *Cercopithecus aethiops var. sabaeus*), whose biological secretions failed to reveal any type of poxvirus other than mpox virus [4]).

The first human case was identified in a nine-month-old infant in 1970 in Zaire, current Democratic Republic of Congo (DRC), in Equateur province [5].

As this case was reported during a period of intensified efforts to eradicate smallpox, the WHO supported an active mpox surveillance program in the DRC from 1981 to 1986 to assess the importance of this new nosological entity [6]. The results of this active surveillance led to the development of a model for predicting human-to-human transmission of mpox. According to this research, mpox had a very low probability of persisting in human populations in the absence of repeated animal contamination. The authors concluded that, at that time, mpox was not yet a major public health threat [6].

However, almost 45 years after the cessation of routine smallpox vaccination, which conferred cross-immunity against mpox, an increase in cases of this disease has been noted in several countries in Africa and outside of this continent in recent years, and this is beginning to constitute a serious public health problem [7]. In May 2022, an unusually high number of mpox cases with sustained chains of local transmission were reported from many countries where the disease is not endemic. This situation prompted the WHO to officially declare mpox a Public Health Emergency of International Concern (PHEIC) on July 23, 2022 [8].

2. Modes of transmission and risk factors

The mpox virus can be transmitted to animals and humans in two modes: (1) animal transmission or primary contamination and (2) human-to-human transmission or secondary contamination. Primary and secondary cases can be infected through

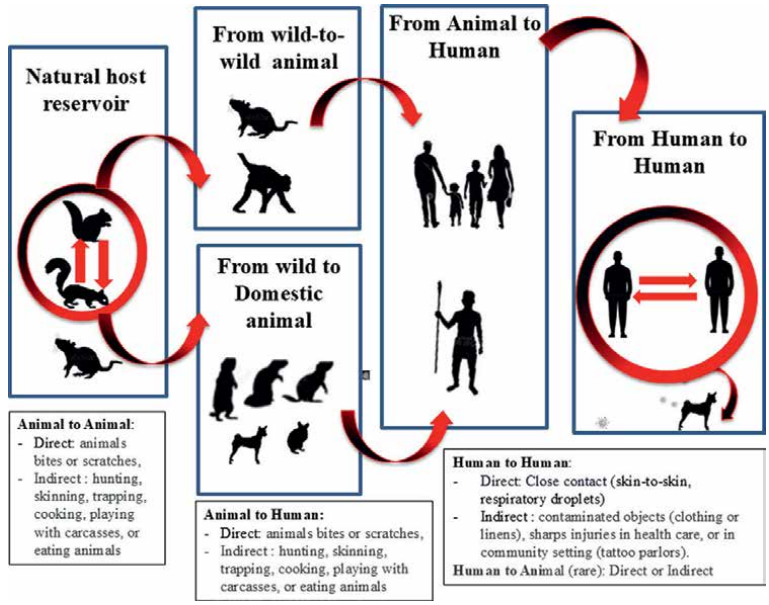


Figure 1.
Modes of MPXV transmission.

direct and close (skin-to-skin) contact with infected humans or animals or through their bodily fluids or fomites (**Figure 1**) [6, 9]. Human-to-human transmission can occur either through inhalation of respiratory droplets or close contact with the body secretions of an infected person or fomites [10] or through vertical transmission during pregnancy (congenital simian orthopoxvirose) [11]. During the 2022 epidemic, the mpox virus circulated mainly among men who have sex with men (MSM), probably through sexual contamination [11].

At the beginning of the mpox emergence, it was observed that animal or primary transmission was predominant and essential and was estimated at 78% in early studies [12]. Today, there is a clear trend toward a change in the mode of transmission of the disease, as the 2022 epidemic was characterized by very high human-to-human transmission, greater than 95% [11].

People living in villages close to dense forests seem to be at greater risk. Subjects at risk are mainly young boys not vaccinated against smallpox in endemic areas, due to the increased susceptibility of this population to the disease. However, the 2022 epidemic showed a higher proportion of older patients who had not been vaccinated against smallpox [13].

3. Geographic expansion in Africa

Since the notification of the first human case in the DRC and prior to the 2022 pandemic, the majority of mpox cases were confined to tropical rainforest areas of the Congo Basin and West Africa [14]. However, in 2005, the geographical distribution of the disease appeared to change, with cases occurring outside the preferred forest zones of Central and West Africa and specifically in Sudan (**Figures 2** and **3**) [15].

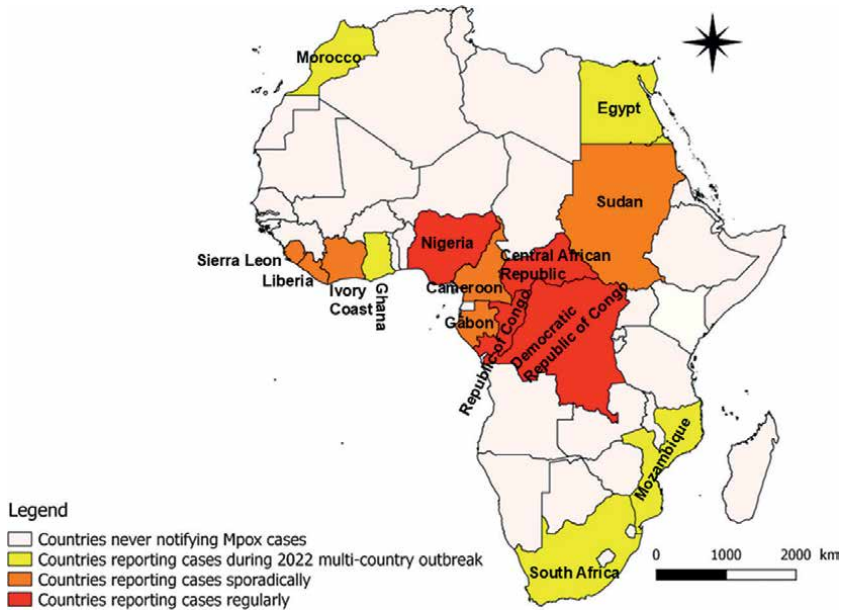


Figure 2.
Countries reporting human monkeypox cases in Africa from 1970 to 2024. For the elaboration of this figure, this work used published sources (research articles, reviews, and WHO reports).

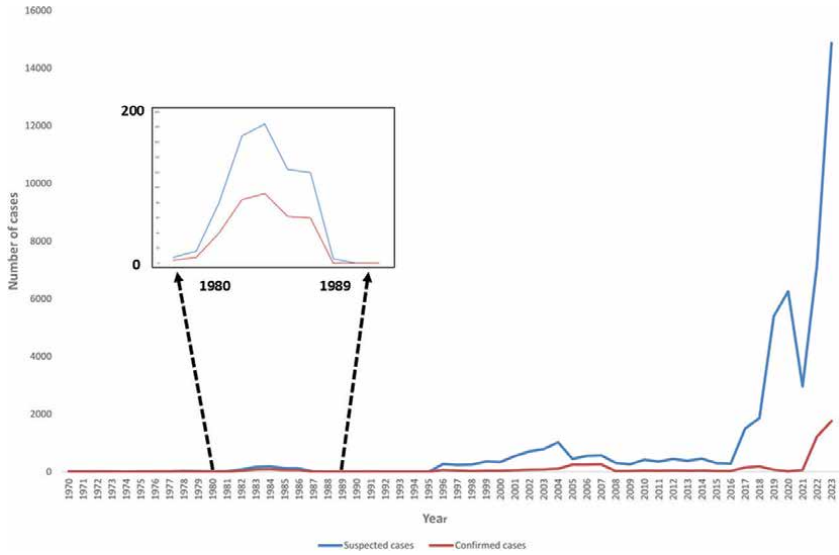


Figure 3.
Timeline of suspected versus confirmed monkeypox cases in Africa, 1970–2023. For the elaboration of this figure, this work used our world in data on https://ourworldindata.org/mpox.

3.1 Mpox of west and Central Africa

In West Africa, human mpox occurred in four countries, namely Nigeria, Ivory Coast, Liberia, and Sierra Leone [14]. Mpox cases appeared in five countries of Central Africa: Cameroon, the Central African Republic, DRC, Gabon, and the Republic of Congo [16, 17].

Between 1970 and 1979, 47 mpox cases were reported in Africa, including 38 in the DRC. Eight patients died, representing a case-fatality rate of 17%. Of these, 83% of patients with clinical signs were children under 10 years of age. Secondary human-to-human transmission was observed in only four cases, representing a secondary attack rate of 7.5% [18].

Between 1970 and 1986, 404 new cases of mpox were reported through the WHO's active mpox surveillance program (1981–1986). Of these, 386 cases of 404 were reported from the DRC, of which 338 cases came from regions where intensive epidemiological surveillance had been set up. Two hundred and forty-five of these three hundred and thirty-eight cases were associated with animal-to-human (zoonotic) contamination, giving a primary transmission rate of 72%. Children were the most affected age group (86% aged under 10, with an average age of 4.4). Deaths occurred in one in ten cases, well below the 17% observed prior to 1980 [6].

From 1986 (the end of the WHO's intensive surveillance program) to 1992, 13 cases of mpox were recorded in Central Africa, namely in Gabon, Cameroon, and the DRC. Between 1993 and 1995, no cases were reported, probably due to a poor mpox surveillance system during this period. Between 1987 and 1995, thirteen cases were documented, including six in 1987, one in 1990, five in 1991, and one in 1992. Of these thirteen patients, one was reported in Cameroon, ten in Gabon, and two in the DRC. These cases involved children only. It was also found that no cases were notified between 1993 and 1995 [19].

Between February 1996 and October 1997, the DRC recorded its largest-ever mpox epidemic. The epidemic was reported in the Katako-Kombe and Lodja health zones (Sankuru health district, Kasai Oriental province, DRC). A total of 511 suspected cases were identified, with mortality ranging from 1.5–3%. Men were most affected (58%), and the epidemic mainly concerned children under 16. This epidemic was marked by substantial human-to-human transmission, estimated at 78% of cases. Nevertheless, the low proportion of cases confirmed biologically and the low case-fatality rate observed suggested that a good number of cases could be attributed to varicella, the clinical features resembling that of mpox [20].

Between January 1998 and December 2002, the DRC reported 31 mpox patients [21], while the Central African Republic reported four cases in 2001 [22]. During the same period, a total of 1265 suspected cases of MPX were reported by the DRC Ministry of Public Health, of which 565 (44.6%) were aged between 10 and 24 years [23]. Only 41% of these cases were biologically confirmed among the 215 samples tested. From January 1, 2001, to December 31, 2004, the DRC's passive disease surveillance system recorded 2734 suspected cases of mpox. Of these cases, 380 were notified in 2001, 545 in 2002, 783 in 2003, and 1026 in 2004 [24].

From November 2005 to November 2007, a total of 760 biologically confirmed cases of mpox were reported in nine health zones of the Sankuru district through the active mpox surveillance project piloted by the University of Kinshasa School of Public Health, the University of North Carolina, and the US Army. Affected subjects had an average age of 11.9 years, and the majority (92.1%) were born after 1980, the period corresponding to the cessation of smallpox vaccination, which conferred cross-immunity against mpox [7].

In 2010, the Republic of Congo reported 8 suspected cases and 2 confirmed cases in the Likouala department [25], while the Central African Republic reported two confirmed cases [26]. From 2010 to 2014, passive surveillance by the Ministry of Public Health revealed that more than 2000 suspected cases of mpox were recorded each year, mainly in the Equateur and Kasaï Oriental provinces [27].

In 2014, 40 years after the last outbreak, an mpox epidemic occurred in Sierra Leone. During this outbreak, only one case was biologically confirmed [28]. Between December 2015 and February 2016, the Central African Republic reported 12 cases of mpox in the Bangassou and Mbomou provinces [29]. In August 2016, the Central African Republic also reported 26 suspected cases of mpox, including 3 confirmed cases in the provinces of Basse-Kotto and Haute-Kotto in Ref. [30]. Between September 2014 and February 2016, data from the DRC Ministry of Public Health notified 587 suspected cases of mpox [31].

From January to August 2017, an epidemic of 88 suspected cases of mpox, including 7 confirmed cases, had occurred in the Republic of Congo, and more specifically in the Likouala department [24]. In 2017, the Central African Republic recorded two epidemics. During the first outbreak, a total of 47 suspected cases (7 confirmed) were reported in February in Mbomou province [24], and during the second outbreak, a total of 3 cases (1 confirmed) were reported in April in Mbaki district [24]. During the same year, the Pujehan district in Sierra had reported a single isolated case of biologically confirmed mpox [24].

From September 2017 to August 2018, Nigeria recorded the largest mpox epidemic West Africa had ever seen. This epidemic occurred in 26 states, and 262 suspected cases, including 113 confirmed cases, were reported. Seven of the reported cases died, representing a case-fatality rate of 6.3%. The epidemic mainly

affected men (75%), the majority of whom were aged between 21 and 40, with a median age of 30 [32].

Between November and December 2017, Liberia had reported a total of 16 suspected cases, of which 2 were confirmed. This epidemic came 40 years after the last outbreak, which this country had experienced [24].

From the first to the 24th week of 2018, data from the Ministry of Public Health in the DRC had reported 2845 suspected cases of MPX [24]. In 2018, from March 17 to April 24, the Central African Republic reported 20 suspected cases of mpox, 9 of which were confirmed [24]. From March 30 to May 30, 2018, Cameroon had reported 16 suspected cases of MPX, including 1 confirmed [24].

In 2019, Cameroon recorded 1 confirmed mpox case in Ekondo Titi province, while the Central African Republic reported 18 suspected cases, of which 14 were confirmed in Lobaye and Ouaka provinces [33]. During the same period, a total of 5288 suspected cases of MPX were reported by the DRC Ministry of Public Health, of which 107 were deaths [33]. In the same year, Nigeria notified a total of 98 suspected cases, of which 47 were confirmed in 11 provinces; the Republic of the Congo reported 2 confirmed mpox cases in Gambona province, and Sierra Leone recorded 1 confirmed mpox case in Kailahun province [33].

In 2020, Cameroon reported 2 confirmed mpox cases in Ayos and Doumé provinces, while the Central African Republic notified 2 suspected cases and 8 cases in Mbomou, Sangha, and Mbaéré provinces [33]. In the same period, passive surveillance by the DRC's Ministry of Public Health revealed that more than 6216 suspected cases of mpox were recorded [33]. During the same year, Nigeria reported a total of 35 suspected cases, of which 8 were confirmed in Delta, Lagos, Plateau, Ebonyi, and Rivers provinces [33].

In 2021, Cameroon notified 4 confirmed mpox cases in Ayos and Nkambé provinces. During the same year, the DRC's passive disease surveillance system recorded 2841 suspected cases of mpox. In the same year, Nigeria reported a total of 98 suspected cases, of which 34 were confirmed in 9 provinces, and Sierra Leone recorded 1 confirmed mpox case in Koinadugu province [33]. From November 2021 to January 2022, the Central African Republic reported 42 suspected cases of mpox, 14 of which were confirmed. This outbreak occurred in Bania, Mambéré Kadéi prefecture, and the secondary attack rate was 59.5% (25/42) [34].

3.2 Mpox outbreak in Sudan

In October 2005, it was the first time that mpox occurred outside its preferred zone in the forests of Central and West Africa. Indeed, between September and December 2005, an MPX epidemic had been reported in Sudan's Unity State (now South Sudan), which is characterized by a semi-arid sub-Sahelian environment. A total of nineteen confirmed or probable cases were reported in the villages of Nuria, Rubkona, Wang Kay, and Modin. Nearly half (52%) of the cases were women, and the majority (80%) were under 20 years of age, ranging from 8 months to 32 years. No deaths were recorded. Although the cause of this epidemic was initially attributed to a new virus derived from the Central African strain [15], Nakazawa et al. demonstrated that the virus responsible for this Sudanese epidemic was genetically close to that of the northern DRC, casting doubt on the hypothesis of a new strain of mpox virus. The probable cause of this epidemic would be either the importation of animals from this northern part of the DRC to southern Sudan and/or a displacement of mpox-infected populations, taking into account the socio-political instability experienced by the region at the time of the epidemic and up to then [35].

4. Mpox outside of Africa

Outside of Africa, historically, there have been a handful of MPXV outbreaks involving animals in Europe and the United States with an uncertain origin of the disease [3]. To add more, human cases of mpox exported outside of Africa have been rare. The first human cases of mpox observed outside Africa were in 2003 during the epidemic in the US due to infested rodents imported from Africa [36].

There is a current trend toward wider geographical distribution of mpox epidemics. In recent years, MPX has spread outside Africa, notably to North America, Europe, and Asia. This extension is linked to the large-scale circulation of MPXV-infected people and the transport of naturally infected animals. In addition, this unexpected extension of the disease outside MPX's preferred African forest areas may be the result of the increased mobility of people and trade within and outside the African continent (**Table 1**).

4.1 First escape: Out to the USA

In May 2003, cases of MPX were reported in the state of Wisconsin. The disease was introduced to the USA by a shipment of 800 diseased exotic rodents imported from Ghana, including giant Gambian rats, African dormice, and ground squirrels. These diseased rodents contaminated the local prairie dogs housed alongside them in

Important events	Country	Time frame	Suspected/ confirmed cases	References
Isolation and characterization of mpox virus	Denmark	1958	—	[3]
Identification of the first human case in country	DRC (former Zaire)	1970	1/1	[5]
Active surveillance program started	DRC (former Zaire)	1981–1986	386/386	[6]
First in country large outbreak	DRC (former Zaire)	1996–1997	511/–	[20]
First outbreak outside Africa	USA	2003	82/47	[37]
First in country outbreak outside the tropical rain forest of Central and West Africa	South Sudan (former Sudan's Unity State)	2005	37/19	[35]
Re-emergence 40 years after silent phase	Sierra Leone	2014	1/1	[28]
Largest outbreak in West Africa	Nigeria	2017–2018	262/113	[32]
First in country outbreak	UK	2018	4/4	[38]
First in country imported case	Israel	2018	1/1	[39]
First in country imported case	Singapore	2019	1/1	[40]
Multi-country outbreak	117 countries	05/2022 to date*	/92030	[41]
First imported case in Sweden	Stockholm	2024	1/1	**

**by December 2023; DRC, Democratic Republic of Congo; UK: United Kingdom; USA: United States of America; **Ref. [42].*

Table 1.
Important events observed during mpox outbreaks between 1959 and 2024.

sales outlets. These infected animals transmitted the disease to the people who bought them through bites, scratches, or contact with their purulent secretions. The epidemic began in Wisconsin and spread to Indiana, Illinois, Missouri, Kansas, and Ohio. A total of 71 cases were reported, of which almost 40% were biologically confirmed. Patients ranged in age from 3 to 43 years, and both sexes were equally affected. No deaths were reported (**Figure 4**) [37].

4.2 Unexpected expansion

4.2.1 MPX outbreak in the UK

Two confirmed cases of MPX were reported in September 2018 in the United Kingdom (UK) in the towns of Cornwall and Blackpool, respectively. The two patients, although from Abuja in Nigeria, where an epidemic of MPX had occurred since September 2017 until that date, did not come from the same chain of contamination. These were the first cases of MPX to be recorded in Europe. One of these patients had transmitted the disease to a healthcare professional. Among the 134 potential contacts of this healthcare worker, the disease had only been transmitted to 4 people. No deaths were reported [38].

4.2.2 A case of imported MPX in Israel

A confirmed case of MPX was reported in Jerusalem, Israel, in October 2018. This case had been imported from Rivers State in Nigeria by a 38-year-old Israeli resident, who had frequented this region in which MPX was actively circulating. This was the first time MPX had reached the Asian continent. The patient did not die, and no cases of secondary contamination were reported [39].

4.2.3 A case of imported MPX in Singapore

In May 2019, Singapore notified a confirmed imported case of MPX. The patient was a man of Nigerian origin who had visited Singapore. The patient did not die. There was no evidence of secondary infection among the patient's contacts [40].

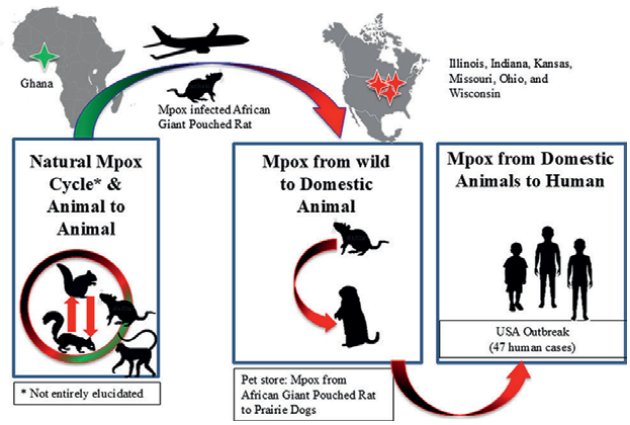


Figure 4.
Mpox out of America.

5. The twenty-first century mpox pandemic

The current mpox pandemic is believed to have originated from a source of MPXV Clade II from Nigeria, which occurred in May 2022 during the travel of an infected Nigerien resident to the United Kingdom. However, several introductions of viruses from Nigeria were also observed between 2017 and 2022 in the United Kingdom, Israel, Singapore, and the United States. Ultimately, one can consider that the actual pandemic which was the largest MPX epidemic in West Africa in history, due to the MPXV Clade II, spread in a pandemic manner, will have started in Nigeria in September 2017 [43]. Therefore, from the point of view of the circulation of MPXV in Africa, we can consider that Clade II actively circulates in West Africa in a human-to-human epidemic manner and that MPXV Clade I circulates independently in an endemic-enzootic manner in Central Africa [41].

5.1 A brief history of mpox emergence

Since it was first identified in laboratory monkeys kept for research in Denmark in 1958, the first human case of MPXV infection was recorded in DRC in 1970 and marked the recognition of mpox as a potential human disease. In the 1980s, human mpox cases were confined to remote areas of the rainforest regions of Central and West Africa. Furthermore, WHO reported for DRC two large outbreaks of hundreds of human cases in 1986 and in 1996; both events underscored the endemic nature of mpox in this region and its potential to spread more widely [15].

5.2 Re-emergence and global spread of mpox in the twenty-first century

In 2003, the first monkeypox outbreak outside of Africa was reported in the United States and spread among 6 states of the country with 71 human mpox cases. It was traced to pet prairie dogs that had been housed with imported infected African rodents from Gambia. This incident highlighted the risk of mpox global spread through the exotic pet trade [37].

After nearly 40 years without reported mpox human cases in the country, Nigeria experienced a significant monkeypox outbreak from 2017 to 2019 due to the MPXV West African Clade II, with over 146 confirmed and suspected cases [32].

Then, in 2021, mpox cases, linked to travelers from Nigeria, were reported in the United Kingdom, the United States, and Singapore [33]. These cases then indicated continued transmission in West Africa with, in fact, the beginnings of international spread. Indeed, a year after, a significant outbreak occurred globally. In early May 2022, an increasing number of human monkeypox (mpox) cases were reported in mpox non-endemic regions of multiple countries across Europe, the Americas, and Asia [44]. Ultimately, in the wave of COVID-19, the widespread nature of this outbreak led to increased public health measures and vaccination campaigns in affected areas. Since May 2022, when WHO declared the mpox outbreak a Public Health Emergency of International Concern (PHEIC), human mpox has emerged with nearly one hundred thousand cases confirmed from more than one hundred countries [13]. Indeed, by December 2023, there had been 92,030 confirmed cases reported in 117 countries.

While at-risk groups and the transmission dynamics of mpox mostly outside of Africa were due to the MPXV Clade II and revealed that more than 99% of reported

cases occurred among men having sex with men with a case-fatality rate (CFR) of <0.1%, the ongoing MPXV Clade I resurgence in several African countries showed only 2133 cases from January 2022 to December 2023, but with a higher case-fatality rate of 1.4 to 11%, mostly in young children (**Figures 5**–**7**) [45].

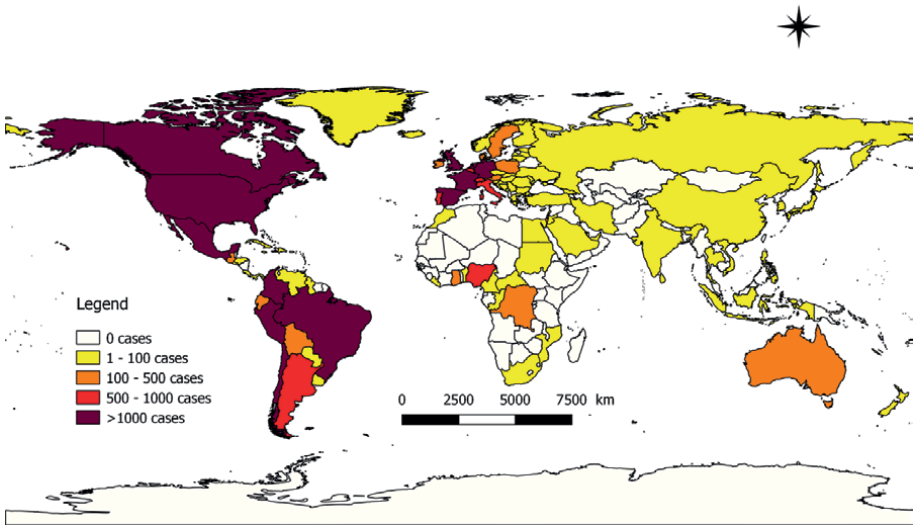


Figure 5.
Worldwide mpox situation during the pandemic from May to December 2022. For the elaboration of this figure, this work used our world in data on https://ourworldindata.org/mpox.

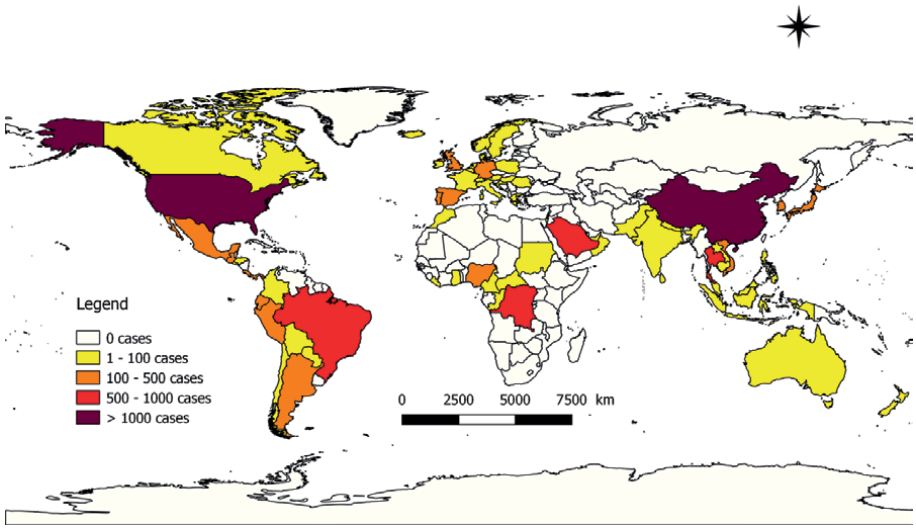


Figure 6.
Worldwide mpox situation during the pandemic from January to December 2023. For the elaboration of this figure, this work used our world in data on https://ourworldindata.org/mpox.

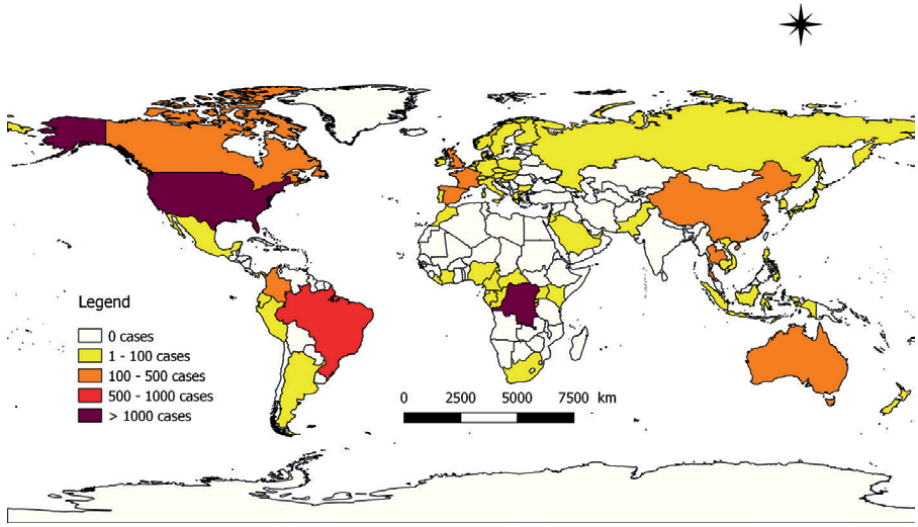


Figure 7.
Worldwide mpox situation during the pandemic from January to August 2024. For the elaboration of this figure, this work used our world in data on https://ourworldindata.org/mpox.

6. The monkeypox virus's natural history

The MPXV comprises two distinct genomic groups, which are the West African Clade II and the Congo Basin clade I. These genomic groups have distinct and overlapping geographic distributions, with each having specific clinical and epizootological characteristics [46].

Today, the epidemiological pattern of mpox is changing with the global outbreaks, and the rising incidence of MPVX infections among young adults in the historical African endemic zones might be a result of the cessation several decades ago (1979) of the smallpox vaccine [47].

6.1 Virus natural reservoir(s) and hosts

When the infection of humans and monkeys appears to be fortuitous, the natural wild animal reservoir of MPXV remains to be confirmed. Although notable progress has been made on the knowledge of hosts and reservoirs of the MPXV, several gray areas persist: Numerous serological surveys have shown that many animals can be infected in nature.

Until now, MPXV has only been isolated from the Thomas' rope squirrel (*Funisciurus anerythrus*) and the sooty mangabey (*Cercocebus atys)*; specimens from giant rats (*Cricetomys emini*) have also shown the presence of anti-Orthopoxvirus antibodies. In addition, several preliminary studies had consistently shown that certain monkey species from tropical Africa possessed anti-orthopoxvirus antibodies and/or MPXV-specific antibodies, such as the following species: cynomolgus monkeys (*Macaca fascicularis*); grivet *Cercopithecus aethiops*; lesser spot-nosed monkey (*Cercopithecus petaurista*); western red colobus (*Piliocolobus badius*), Mona monkey

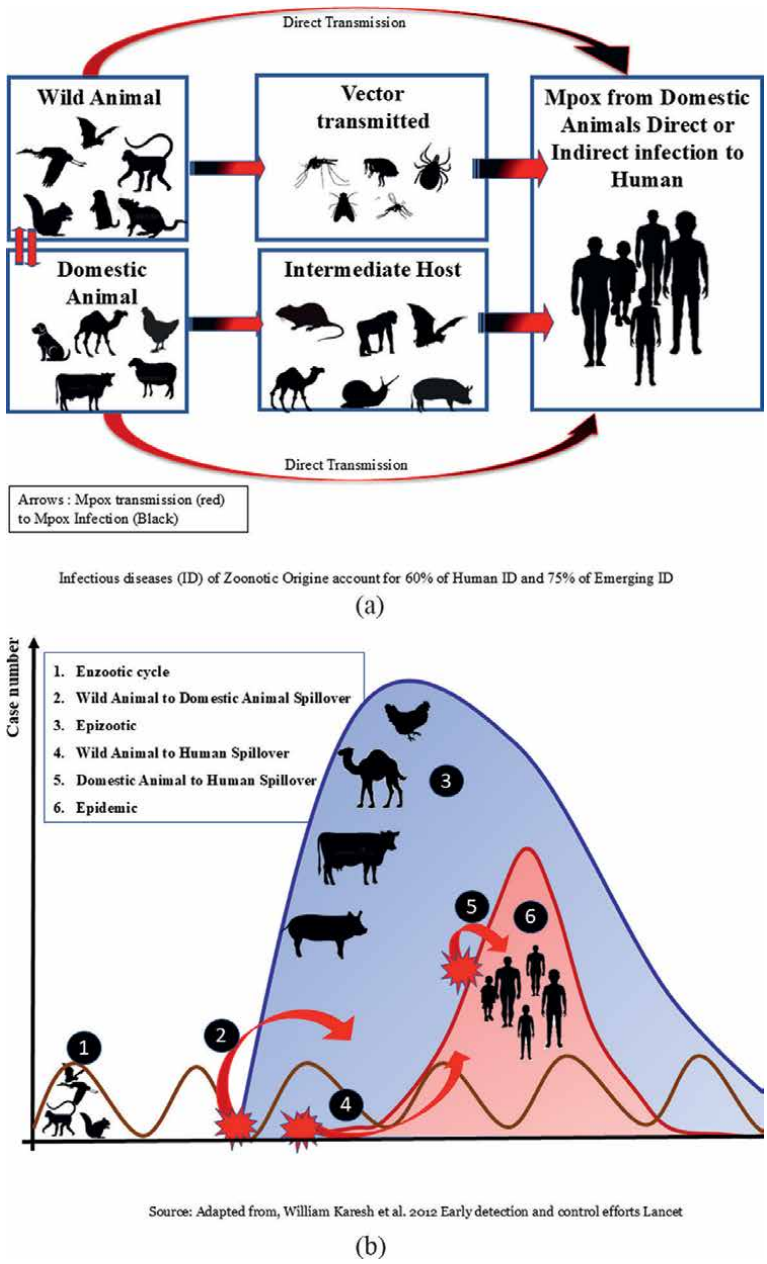


Figure 8.
a. Zoonotic Disease Spread, the Actors. b. Zoonotic Disease Spread, the Mechanisms.

(*Cercopithecus mona*); Diana monkeys (*Cercopithecus diana*) [48, 49]. Rodents and other small mammals have also been suspected to be naturally infected, including the African dormice (*Graphiurus spp.)*; the tetradactyl horn rat (*Petrodromus tetradactylus*); chimpanzee (*Pan troglodytes*); red-legged squirrel, *Heliosciurus rubobrachium;* Congo rope squirrel (*Funisciurus congicus*) [50–52].

However, the primary reservoir of the MPXV remains unknown. From the original hypothesis of an exclusive simian reservoir (*Macaca fascicularis*) at the origin of the

discovery of MPXV [52], we have moved on to the hypothesis of a more diversified host-reservoir chain of transmission, through primates as secondary hosts of the MPXV transmitted from squirrels (*Funisciurus anerythrus*), from which the MPXV has so far been isolated in nature when several monkey species are known to prey on squirrels (**Figure 8a, b**) [28].

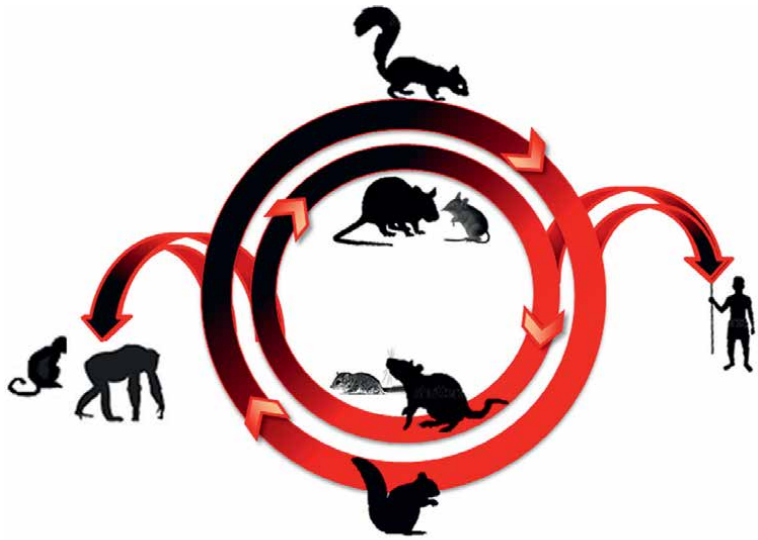
6.2 Understanding the natural cycles

The MPXV follows natural cycles that primarily involve interaction and zoonotic transmission between animal hosts and humans. We can thus distinguish two transmission cycles: a natural transmission cycle, which involves one or more natural animal hosts and accidental transmission (spillover) to humans; and an inter-human cycle, in which the virus becomes human and exercises its potential for intra-species transmission (**Figure 9**).

6.2.1 Natural cycle of transmission: The sylvatic cycle (forest cycle)

The sylvatic cycle is the natural cycle of monkeypox virus, which occurs mainly in the tropical forests of Central and West Africa. This cycle involves interactions between different animals, mainly rodents, which are the main natural known reservoirs of the virus (Rodents such as rope squirrels, Gambian squirrels, dormice, and African forest rats harbor the virus).

The natural reservoir hosts (primarily rodent squirrels) of MPXV are infected and develop viremia and skin lesions, which are still little documented in most suspected species. However, MPXV infecting animals suspected as a reservoir vector of the



Legend: The natural reservoir of Mpox and therefore the natural cycle of the virus remains unknown. However Squirrels of the genera *Funisciurus* and *Heliosciurus* as well as Rodents of the genera *Cricetomys*, *Graphiurus*, and *Petrodromus* appear to be related to the natural cycle. Also, frequently monkeypox virus has been identified from non-human primates' chimpanzees (*Pan troglodytes verus*) and monkey (sooty mangabey).

Figure 9.
Natural mpox cycle.

virus does not seem fatal, and horizontal intra- and inter-species transmission seems the most likely mechanism to hypothesize [53]. From the transmission point of view, the squirrel's potential reservoir of the virus has experimentally shown its capacity to develop viremia, infectious skin lesions, and possible inter-species transmission via respiratory droplets or other biologically infected products.

6.2.2 Domestic cycle of transmission: Human-human transmission

The domestic cycle occurs when the monkeypox virus passes from animal reservoirs to humans and then enters a human-to-human domestic cycle. This cycle involves several modes of transmission and can lead to human epidemics and the ongoing pandemic. Human-to-human transmission occurs by: (1) Direct Contact: Touching skin lesions, bodily fluids, or respiratory secretions of an infected person. (2) Contaminated Objects: Use objects contaminated by the bodily fluids of an infected person, such as clothing, sheets, or utensils [6, 9]. (3) Respiratory Droplets: Transmission by respiratory droplets emitted during coughing, sneezing, or talking [10]. Human index case contracts the virus through direct contact with bodily fluids, skin lesions, or respiratory secretions of infected animal or human. It can also occur from handling or consuming infected bushmeat.

Once a human is infected, the virus can spread from person to person through close contact with bodily fluids, skin lesions, or respiratory droplets from an infected person [6, 9].

Although it was less common than zoonotic transmission, as for today's pandemic, human-to-human transmission occurs much more frequently and is often facilitated by close, prolonged contact. Mpox can spread through close contact of any kind (kissing, touching, oral, and penetrative vaginal or anal sex) with someone who is infectious. Additionally, in September 2018, the virus was transmitted from a patient to a healthcare worker in the United Kingdom, probably through contact with contaminated bedding [38]. Also, international travel and human mobility can introduce MPXV to new non-endemic (i.e., not zoonotic) regions, where it can spread locally

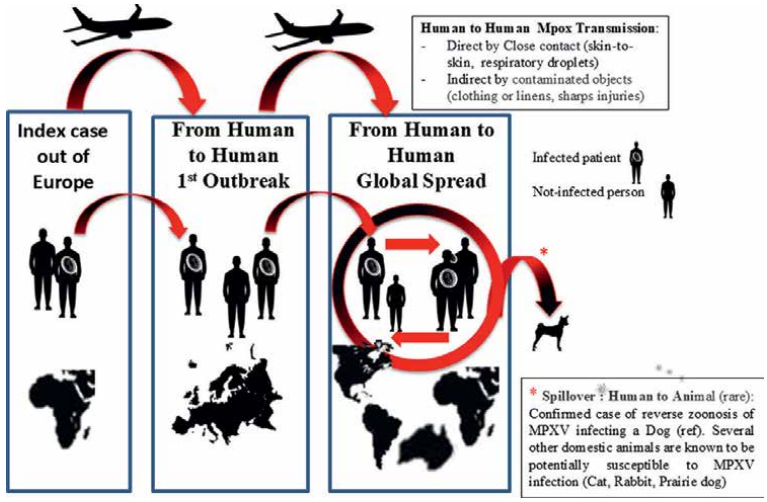


Figure 10.
Domestic human-to-human cycle.

if control measures are not put in place. In conclusion, the monkeypox virus mainly follows two cycles: the sylvatic cycle, which takes place among animal populations in tropical forests, and the domestic cycle, where the virus passes from animals to humans and can spread between humans. Understanding these cycles is essential for controlling and preventing monkeypox outbreaks, particularly in regions where MPXV is endemic (**Figure 10**).

7. Pandemic preparedness and readiness

7.1 Pandemic response

The areas of action for preparedness and response to the mpox pandemic include the priority areas of public health response, surveillance, community participation, and international support. The first response involves rapid diagnosis for the management of suspected or confirmed infected patients. Efforts to track and diagnose MPXV have improved over the years. Laboratory capacity to identify the virus has expanded, enabling quicker responses to outbreaks.

Immunization: The smallpox vaccine, which also provides protection against monkeypox, has been used in outbreak response efforts. The development and distribution of newer vaccines specifically targeting monkeypox are ongoing.

Education and Prevention: Public health campaigns have focused on educating communities about the risks of zoonotic transmission, the safe handling of animals, and the importance of vaccination.

Monkeypox remains a significant public health concern, particularly in regions where it is endemic. Increased global travel and trade have heightened the risk of international spread, making surveillance and preparedness critical. The history of monkeypox highlights the importance of understanding zoonotic diseases and their potential impact on global health.

7.2 Pandemic lessons: Forecasting the risk

Lessons from the pandemic. If the global mpox epidemic does not reach the scale of that of COVID-19, it also carries valuable lessons for preparation for future pandemics. Beyond the years of forgetting research on MPXV, then endemic for decades but only in Central Africa, the development of this mpox pandemic in the global north was a demonstrative element for the approach of global health, which today requires and is required to consider the risk of emergence due to zoonotic diseases on all continents with active surveillance.

7.2.1 Lessons learned and recommendation

The mpox pandemic revealed major shortcomings and thus provided some major lessons that must be followed, such as early detection of the index case and the area of emergence followed by a rapid response (contact tracing); reasoned confinement (isolation, quarantine) with communication (clear and coherent) and education of the general public (precise information on the disease, its transmission, and preventive measures); and the implementation of surveillance of populations at risk will be possible if there is targeted and dynamic health infrastructure and allocation of resources (supplies and training of health professionals).

7.2.2 Cooperation and partnership

The mpox situation showed how international cooperation could lead to an effective fight against the disease with an early exchange of data essential for diagnosis, the development of vaccines, and treatments. The rapid distribution of vaccines enabled local and then global control of the pandemic and demonstrated the importance of flexible multiple platforms for the rapid development of medical countermeasures.

What is more, for good management of epidemic risk, MPXV epidemics have essentially underlined the importance of equitable access to health services, vaccines, and treatments.

At national and international levels, coordination of rest was also essential and remains so; for example, the establishment of multi-sectoral coordination structures such as the National Institute of Public Health with Public Health Emergency Operation Centers.

Finally, and this was launched for the Ebola virus fever epidemic in 2014, a Pandemic Fund was launched by the World Bank.

7.2.3 The exercise of biosurveillance

Health surveillance, long neglected in endemic areas, often intertropical, and their populations at risk, today requires the implementation of a holistic approach within the well-defined framework of a One Health approach considering shared environments between humans (communities and individuals), hosts (domestic and wild), and potential reservoirs/vectors of the incriminated pathogen. This biomonitoring must also be carried out in the territories and at the borders with community participation and the detection (genomic and metagenomic) of potentially pathogenic agents. The mpox pandemic, like that of COVID-19, cruelly demonstrates the persistent global threat posed by the agents responsible for infectious diseases. By learning from these and past outbreaks, we can improve preparedness and response

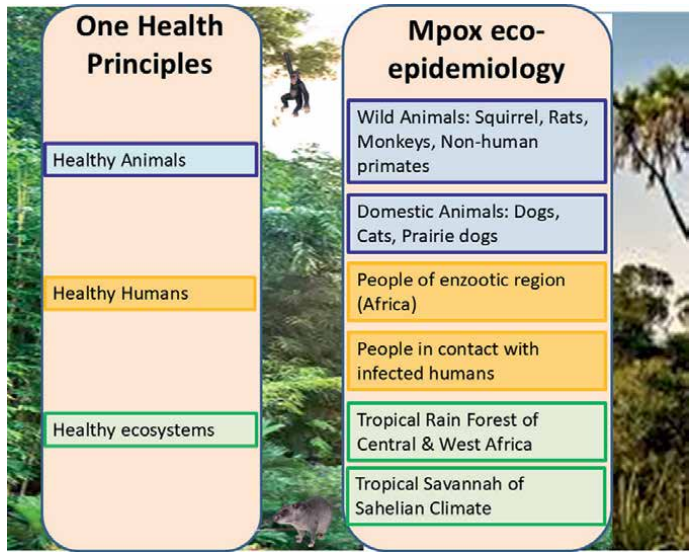


Figure 11.
One health and pandemic treaty.

strategies, eliminating the risk and/or mitigating the impact of future threatened outbreaks. After this double experience of the pandemics of the twenty-first century, enlightened actions are underway, such as the economic management of the risk and the proper resolution of the mpox epidemic [54, 55]. Accordingly, global risk awareness and the emerging Pandemic Treaty support the One Health approach that must be applied with veterinary and public health intervention teams trained specifically in zoonotic risk and multi-sectoral participation driven by environmental health (**Figure 11**) [56].

8. Conclusion

The risk of MPXV re-emergence remains a major concern, particularly in the context of global trade and increased interconnectedness of populations. Although previous epidemics were relatively contained, thanks to the pre-existing smallpox vaccine, the human-to-human transmission observed in 2022 showed that this virus, which has long remained endemic and epizootic in Africa, can now spread beyond its original borders. It is well known that zoonoses, such as MPXV, are closely linked to human interactions with wildlife and environmental changes, which increase the opportunities for viruses to pass between species. Thus, deforestation, rapid urbanization, and increased contact with animals carrying the virus amplify this risk. But human-to-human transmission exists and favors the rapid expansion of this virus in high-density populations. Thus, to prevent a new large-scale emergence, several measures must be put in place. First, it is crucial to strengthen epidemiological surveillance, particularly in endemic/enzootic regions and those that have already experienced epidemic outbreaks. Rigorous monitoring of human and animal cases, as well as early detection measures, would make it possible to rapidly contain any resurgence. However, the increasing mobility of humans is a factor in the spread of the virus that is difficult to control except during cross-border travel. Second, research into more effective vaccines and specific treatments must be intensified. While smallpox vaccines have shown some efficacy, better solutions adapted to this disease are needed. In parallel, increased awareness among the general public and health professionals is essential to recognize the symptoms and take appropriate measures, particularly when traveling to risk areas. Finally, it is essential to implement strategies to reduce contact between humans and wildlife through stricter environmental policies and responsible management of natural habitats. These combined actions can significantly reduce the likelihood of an uncontrolled re-emergence of monkeypox.

Acknowledgements

This manuscript has been developed with several unpublished materials of B-A Manja's doctoral thesis done at the University of Bourgogne Franche-Comté and Kinshasa University; therefore, we would like to deeply thank all directors and supervisors of the thesis: Jean-Jacques Muyembe, Frédéric Mauny, Didier Bompangue, and Pascal Handschumacher.

We would like to extend our warm acknowledgment to the members of the Research and Training Unit on the Ecology and Control of Infectious Diseases of the University of Kinshasa and all the members of the Clinical Methodology Center of the Teaching Hospital Center of Besançon, France, for their constant support.

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Section 2

Clinical Aspects, Diagnostics and Miscellaneous

Chapter 6

Perspective Chapter: Gastrointestinal Manifestations of Mpox Infection

Dawit Jowhar, Christian Salcedo, Hayes Walker and George N. Verne

Abstract

Cases of Monkeypox virus infection (Mpox) were initially relegated to Central and West Africa; however, in May 2022, outbreaks began to occur in non-endemic areas. Patients with Mpox infection can present with several symptoms in the gastrointestinal tract, such as abdominal pain, proctitis, and hematochezia. While several case reports have been published that show the presentation and management of patients with M. pox, it is fundamentally essential to have a collective resource for gastroenterologists to identify patients with this infection and appropriately manage them to ensure a safe outcome. The purpose of this book chapter is to present the variety of gastrointestinal manifestations that can present in patients infected with Mpox and the best way to manage them appropriately. We will present the current state of knowledge about gastrointestinal manifestations of Mpox by analyzing key studies that have been published to date and provide a broad overview of the subject as it pertains to gastroenterologists.

Keywords: Mpox, monkey pox, abdominal pain, proctitis, hematochezia, rectal pain, tenesmus, gastrointestinal symptoms

1. Introduction

Monkeypox virus (Mpox) is a zoonotic pathogen that belongs to the Orthopoxvirus genus of the Poxviridae family. It was first isolated and identified in 1958 during an outbreak in monkeys from Singapore in a laboratory in Denmark. The first human case of monkeypox was identified in the Democratic Republic of Congo in 1970. Mpox is divided into two distinct clades, based on where it is endemic: the Congo Basin (Central African) and the West African clade. The Congo Basin clade is associated with more severe diseases with higher mortality rates (10%) than the West African clade (4%). The increase in monkeypox cases outside these endemic regions highlights the need for improved surveillance and response strategies. The fact that

most cases of Mpox occur in rural Africa means that there is significant underreporting of infections with this pathogen, and thus a considerable underestimation of its potential threat to the global population [1, 2].

The monkeypox virus is believed to be derived from other orthopoxviruses, particularly the variola virus, which is the causative agent of smallpox, as well as the cowpox virus. The virus itself is relatively large (200–250 nm), brick-shaped, and surrounded by a lipoprotein envelope with a linear double-stranded DNA genome. It relies on host ribosomes for mRNA translation but retains all other necessary proteins for transcription and replication. The primary reservoir hosts are thought to be rodents. The African giant pouched rat, as well as some other various species of squirrels, have been implicated as potential hosts. Increasing genetic diversity and variability of the monkeypox virus raises concerns regarding its ability to adapt to new hosts and environments, as well as creating more challenges for public health strategies and the development of vaccines. Zoonotic transmission is classically regarded as the primary route through which humans were initially infected, which typically occurs through direct contact with infected animals or exposure to their bodily fluids. This usually occurs through bites, scratches, or consumption of bushmeat. Human-to-human transmission of the monkeypox virus can occur but was typically considered less common. Interestingly, the Congo Basin clade of Mpox has several documented cases of human-to-human transmission compared to the West African clade. The difference in virulence between these two clades can be attributed to variability in genome organization. It was during the 2003 Mpox outbreak in the United States that Mpox received attention as a global public health threat. This was due to a multistate outbreak attributed to close contact with infected prairie dogs acquired as pets, with molecular investigations identifying the West African Clade as a causative source [2–4].

Human-to-human transmission can occur through respiratory droplets, direct contact with lesions on a person infected with Mpox, or through fomites, such as contaminated clothing or bedding. Some studies have shown that bodily fluids, such as semen, saliva, urine, and feces, can have very high viral loads. This suggests that sexual transmission is a major factor in the spread of the monkeypox virus. There is a concern that although human-to-human transmission has been limited in the past, the decreasing herd immunity to orthopoxviruses over time has led to the increasing spread of disease between humans. Behaviors such as increased contact with wildlife and bushmeat consumption have strongly contributed to recent monkeypox outbreaks. Other factors include international travel and the exotic pet trade market. Over the past several decades, sporadic cases have occurred with transmission between people traveling from Nigeria to places such as Israel, Singapore, the UK, Canada, and several US states, including Texas and Maryland. The most significant outbreak outside endemic areas of monkeypox occurred in 2022, with many cases reported in multiple countries, such as the United States, Canada, the United Kingdom, and several other European nations. This Mpox outbreak was declared a global health emergency because of the widespread prevalence of cases [2, 5].

With the observation of these outbreaks, it has become apparent that specific demographic populations are more vulnerable to infections. Men who have sex with men (MSM) are among the most vulnerable groups. Correlating factors and other vulnerable populations include HIV status and people who have not been vaccinated against smallpox, along with any individual with comorbidities that predispose them to weakened immune system responses. Since the discontinuation of routine smallpox vaccination in the 1970s, the apparent prevalence and incidence of Mpox infections have

increased, revealing cross-immunity against Mpox. Studies from almost 600 confirmed cases in Spain in 2022 revealed that 99% of the cases affected MSM. Over 1300 cases were reported in Germany by mid-2022, mainly in the MSM population. Emerging data indicate that these recent outbreaks could be caused by the West African clade of Mpox, while other data suggests that a newly emerging clade could be the culprit. As of January 2023, there were a total of 84,716 confirmed cases [1, 2, 4, 5].

In general, the initial symptoms of infection with monkeypox virus include fever, headache, myalgia, lymphadenopathy, and rash development. Importantly, lymphadenopathy is a key feature of monkeypox infection that can differentiate it from smallpox infection. These symptoms usually follow an incubation period of 7–14 days, with an observed upper limit of approximately 21 days. Most infected patients are contagious during the several days of prodromal symptoms following the incubation period. The rash progresses through several stages (macular, papular, vesicular, and pustular) over 2–4 weeks and ultimately scabs over in most cases. Antibodies are typically detected in the serum when a rash begins to appear. After the rash has scabbed over and the crust has fallen off, patients are no longer considered contagious [2, 5].

In severe cases or those cases involving the vulnerable populations described above, more debilitating manifestations of the disease and complications can arise. In the MSM population, lesions predominantly affect the genital, perineal, and perianal areas. Inguinal lymphadenopathy is another predominant feature suggesting that sexual transmission plays a key role in the spread of Mpox. Patients can also present with several symptoms along the gastrointestinal tract, such as abdominal pain, proctitis, and hematochezia, which will be discussed in greater detail later in this chapter. The differential diagnoses of patients who present with the above symptoms should include smallpox, disseminated zoster, chickenpox, disseminated herpes simplex, measles, syphilis, scabies, rickettsial pox, bacterial skin infections, and drug-associated eruptions. Documented complications of Mpox infection include severe dehydration, sepsis, pneumonia, encephalitis, bacterial superinfection of the skin, permanent skin scarring, permanent corneal scarring, and death [5, 6].

In this chapter, we will present pertinent information about the gastrointestinal manifestations of Mpox covering diagnosis, symptomatology in the gastrointestinal tract, imaging and endoscopy findings, endoscopic precautions, as well as prevention and treatment measures currently in place, using the latest guidelines as well as evidence from case reports, case series, systematic reviews and meta-analysis that have been published in the global literature. Our aim is to serve as a resource for the healthcare team involved in patient management, particularly in the gastroenterology field of medicine.

2. Workup for the Mpox patient

When a patient with suspected Mpox presents in a clinical environment, the differential is broad. Establishing an assessment with a thorough history is key. When completing a physical examination for patients suspected of Mpox, wearing appropriate protective equipment, as outlined in the endoscopy section, is recommended. This ensures protection for both patients and hospital staff. The Centers for Disease Control (CDC) provides guidelines for endoscopy suite precautions and disposal of materials after exposure. A general skin exam can show lesions from the mouth to the anus (oral, perioral, perianal, perirectal, and genital lesions). A study by Thornhill et al. showed that 95% of the patients in the study had skin findings [7].

It is also essential to perform head, eye, neck, cardiac, respiratory, and abdominal assessments. A digital rectal examination may be indicated to appropriately assess for bleeding and rectal lesions when gastrointestinal complaints are voiced; however, this procedure may not be tolerated secondary to pain.

The standard workup for patients should include blood tests and imaging studies. Labs should be tailored according to the patient's presentation. Initial labs can include but are not limited to a comprehensive metabolic panel (CMP), complete blood count (CBC), blood cultures, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), human immunodeficiency virus (HIV), hepatitis panel, rapid plasma reagin (RPR), and Chlamydia/Gonorrhea. When lesions are found, direct swab of the lesions is necessary to confirm the diagnosis. Unroofing the lesions is difficult to do with a swab, given that the lesions are filled with solid material. The use of sharps for unroofing is not recommended because of the risk of infection. The diagnostic test is a polymerase chain reaction of Orthopoxvirus DNA, and two swabs are recommended for each swabbed lesion [8].

Imaging can be used to further assess clinical and laboratory findings. Rectal magnetic resonance imaging (MRI) is recommended for patients presenting with proctitis, especially in cases of rectal perforation and/or obstruction. Abdominal imaging can also be performed using CT or MRI [9].

3. Gastrointestinal symptoms of Mpox

There is a cluster of gastrointestinal symptoms that are present in patients who present with Mpox, as shared in the introduction. Although there is evidence that the most common manifestations of Mpox are skin lesions, several large studies have reported the presence of gastrointestinal manifestations in both upper and lower tracts. We have summarized some pertinent GI findings in the studies listed below.

A systematic review by Ramakrishnan et al. examined several papers and patient profiles across multiple countries. The symptoms included abdominal pain, diarrhea, nausea, painful defecation, proctitis/rectitis (inflammation of the anus or rectal lining), proctalgia (sudden, severe, and episodic anorectal pain due to the potential involvement of anal sphincter spasms), rectal bleeding, rectal/anal pain, rectal perforation, tenesmus (painful and constant urge to defecate despite an empty colon or recent bowel movement), and vomiting. The authors determined that proctitis was the primary gastrointestinal manifestation of Mpox [10].

Thornhill et al. and presented an international case series of 528 patients with Mpox across 16 countries, highlighting the dermatologic and systemic findings. GI symptoms include anorectal pain, proctitis, tenesmus, diarrhea, pharyngitis, and odynophagia. The most common presenting symptoms were rash and skin lesions (95% of patients), pharyngitis (21% of patients), and proctitis/anorectal pain (14% of patients). A total of 97% of the patients had a positive PCR result obtained from the skin/anogenital area. Most encounters were conducted in an outpatient setting. Six% of patients were Hepatitis C antibody-positive and 2% were hepatitis C Virus RNA positive. The three most common places where patients presented in this study were HIV clinics, sexual health clinics, and the emergency department [7].

Liu et al. published a systematic review and meta-analysis by using 77 studies (4 cross-sectional studies that compared symptoms between HIV-infected and HIV-uninfected Mpox patients, and 13 case series with information on HIV status) which they included in the meta-analysis for comparing symptoms between HIV-infected

and HIV-uninfected Mpox patients. Their meta-analysis showed that skin lesions, fever, and lymphadenopathy are the most commonly reported symptoms. Regarding GI symptoms, rectal pain/anal pain/proctitis was reported in 43 studies with a pooled estimated prevalence of 18.5%. Anogenital lesions were the most common type of skin lesions [11].

A cross-sectional study of patients was conducted between May 1 and July 1, 2022, 226 from 18 sites in 15 countries. Rashes or skin lesions were reported in 61% of the patients. A total of 55% reported genital or perianal lesions, 15% reported rectal pain, 6% reported diarrhea, and 4% reported both rectal pain and tenesmus. The authors commented that patients with HIV were more likely to have diarrhea or perianal rash. Nearly all patients in their study were MSM, half of whom had an HIV infection [12].

A systematic review and meta-analysis of gastrointestinal symptoms in Mpox patients was performed by analyzing a total of 31 studies with 9189 Mpox patients. The gastrointestinal symptoms were vomiting (15 studies), diarrhea (11 studies), nausea (10 studies), abdominal pain (nine studies), and anorexia (three studies). The overall pooled prevalence of abdominal pain (9%), anorexia (47%), diarrhea (5%), nausea (10%), and vomiting (12%). The overall pooled prevalence rates of oral ulcers, dysphagia, and odynophagia were 33%, 57%, and 31%, respectively [13].

Yakubovsky et al. reviewed the cases of 70 patients diagnosed with Mpox at the Tel Aviv Sourasky Medical Center. Their criteria for diagnosis of proctitis was meeting at least two of the following criteria: mucopurulent anal discharge, anorectal bleeding, anorectal pain, and anorectal itch, sensation of rectal fullness or incomplete defecation, and tenesmus. A total of 26 patients had signs and symptoms of proctitis. Three patients with proctitis had an anal mass protruding from the rectum accompanied by excruciating pain. Anal pain was the most common proctitis symptom, followed by anal discharge, and pain during defecation. Diarrhea, constipation, and bloody stools were seen in 4, 3, and 2 patients, respectively. Nineteen patients had anorectal lesions and 17 patients had proctitis symptoms preceding the rash [14].

Another study that performed rectal testing in 18 MSM with symptoms consistent with Mpox infection showed rectal Mpox DNA in 9/9 patients with proctitis and 7/9 patients without proctitis. 10/18 patients had HIV [15].

Hatami et al. published a systematic review and meta-analysis of Mpox-confirmed patients by analyzing peer-reviewed publications over the 10 years before and during the 2022 outbreak and their findings. Proctalgia/proctitis was an unprecedented clinical manifestation of Mpox during the 2022 outbreak, with a calculated pooled frequency of 16.6% [16].

A case report on the first two cases of monkeypox infection in humans diagnosed in Germany reported that one of the patients had dysphagia and white spots on the tonsils. Oral lesions appeared prior to the presence of other skin lesions [17].

This is particularly important for gastroenterologists, as they may be consulted to diagnose and treat symptoms of the upper gastrointestinal tract, such as dysphagia or odynophagia, or of the lower tract, such as proctitis. These findings can be helpful in terms of what is expected during endoscopy in patients who present with Mpox.

4. Pertinent history for the gastroenterologist

Obtaining the history of a patient, particularly if they have a history of sexually transmitted infections (STI) such as HIV/AIDS, is important, as several studies have shown this to be a coexisting infection. Pertinent information includes sexual

orientation, HIV status, history of sexually transmitted infections (STI), and foreign travel. For male patients, it is also recommended to obtain a sexual history of MSM. The timing and onset of symptoms can also help to determine whether endoscopic intervention is necessary.

5. Imaging findings of Mpox

Few case reports have shown the imaging results of Mpox using CT or MRI. A case report of Mpox proctitis in a 61-year-old presenting with rectal pain and bleeding showed circumferential thickening of the low to mid-rectum with mesorectal fat stranding and lymphadenopathy on CT imaging [18]. Another report of a 41-year-old male presenting with rectal pain and facial rash showed a CT scan that revealed severe anorectal proctitis with discrete large mural hypoattenuated anorectal ulcers, perirectal fat stranding, pelvic free fluid, and an increased number of small inguinal lymph nodes [9]. A patient in his late 40s who had symptoms of rectal pain in addition to other symptoms underwent MRI of the rectum, which showed active proctitis with enlarged mesorectal space lymph nodes and inflammation of the lower anal canal mucosa at the anal verge [19]. A patient who presented with Mpox in his mid 40s with symptoms of fever, rash and rectal pain underwent MRI performed 5 days after admission, which showed inflammation affecting the mid-to-lower rectum with mural thickening, surrounding edema, and extensive reactive nodal changes within the mesorectum [20]. A retrospective

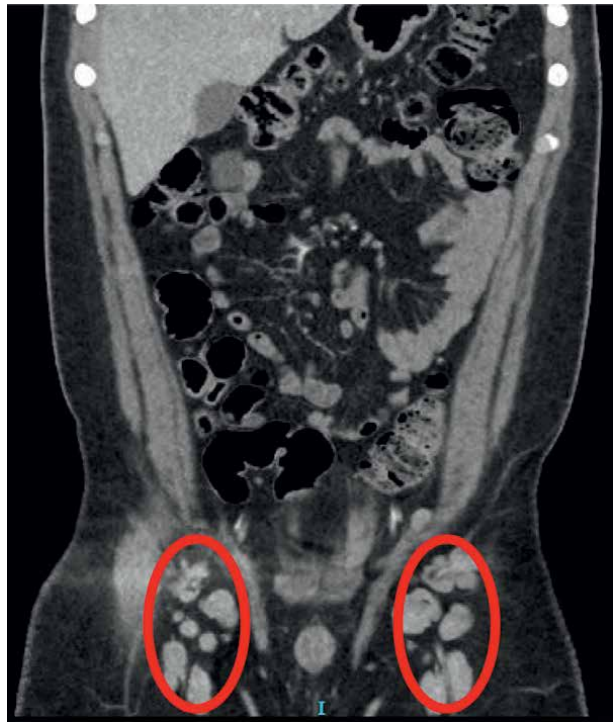

Figure 1.
Cross-sectional CT imaging of a 22-year-old male patient with Mpox. Circled areas highlight adenopathy [22].

study of 21 patients with PCR-positive Mpox who underwent abdominopelvic CT showed varying degrees of rectal thickening, with 17 of 21 patients having abnormal perirectal lymph nodes, and 20 out of 21 showing perirectal stranding (**Figure 1**) [21].

Despite the limited availability of an imaging database, there are a few commonalities in the imaging findings among these patients. These characteristics seen on either CT or MRI include mesorectal fat stranding, lymphadenopathy, presence of ulcers, tract inflammation, and rectal wall thickening. Including these imaging findings in the differential diagnosis can aid in the correct diagnosis of the disease, particularly in patients presenting with proctitis or rectal pain.

6. Endoscopy findings of Mpox

Most publications in current literature provide symptomatic descriptions of Mpox presentations. Few studies have reported endoscopic findings in patients with Mpox. There are a few case reports of upper endoscopy and flexible sigmoidoscopy in the current literature.

Fernandez and Regino reported the case of a patient presenting with skin lesions, fever, sore throat, and severe dysphagia. Upper endoscopy showed ulcerated lesions in the oral cavity and hypopharynx and well-defined ulcers [23].

A case report from Germany analyzed six patients who self-identified as MSM with confirmed Mpox. The chief complaint of most patients was intense anal pain. Three patients underwent proctoscopy, which showed findings such as rectal ulcers, proctitis, and anal ulcers [24].

Another case report by Mavilla et al. examined a patient with Mpox-associated proctitis. The presenting complaint of the 34-year-old male patient with HIV was rectal pain and bleeding. Flexible sigmoidoscopy with biopsy revealed erosions in the distal sigmoid colon and severe proctitis, characterized by deep ulceration and scattered pustular lesions. Rectal biopsies were notable for ulcerated mucosa with viral cytopathic effects [25].

A case report of a 39-year-old HIV-positive male who presented with anal pain, dyspnea, and gastrointestinal bleeding for a 2-week duration showed colonoscopy findings notable for a 3-cm circumferential rectal ulcer with exudate, significant induration, and necrosis. Rectal biopsy revealed ulcerated mucosa with acute proctitis and necrosis. Mpox infection was confirmed using an anti-vaccine virus antibody [26].

An observational study of anal monkey pox disease, which included 65 men with monkeypox anal infection in a single proctology center who underwent anoscopy or rectoscopy performed during the first visit, showed perianal vesicles (n = 24 patients; 36.9%) and ulcerations in the perineal (n = 42; 64.6%), anal canal (n = 28; 43.0%), and rectal (n = 25; 38.4%) localizations. Proctitis was observed in 49 (75.4%) patients [27].

A UK-born male with HIV, aged in his 30s, presented with a 2-day history of rectal pain. Proctoscopy revealed erythematous rectal mucosa with evidence of blood and pus in rectal swabs [19].

Our team in Tennessee performed flexible sigmoidoscopy in a patient with Mpox, which revealed a non-bleeding rectal ulcer, friable rectum and anal canal, micro-abscesses in the rectum and anus, and a rectal polyp (**Figure 2**). Rectal polyp biopsy was notable for focal active proctitis with histio-lymphocytic aggregates and was negative for dysplasia. Anal canal biopsy was notable for colonic-type glandular mucosa with cryptitis, ulceration, granulation tissue, and reactive epithelial changes (**Figure 3**) [22].

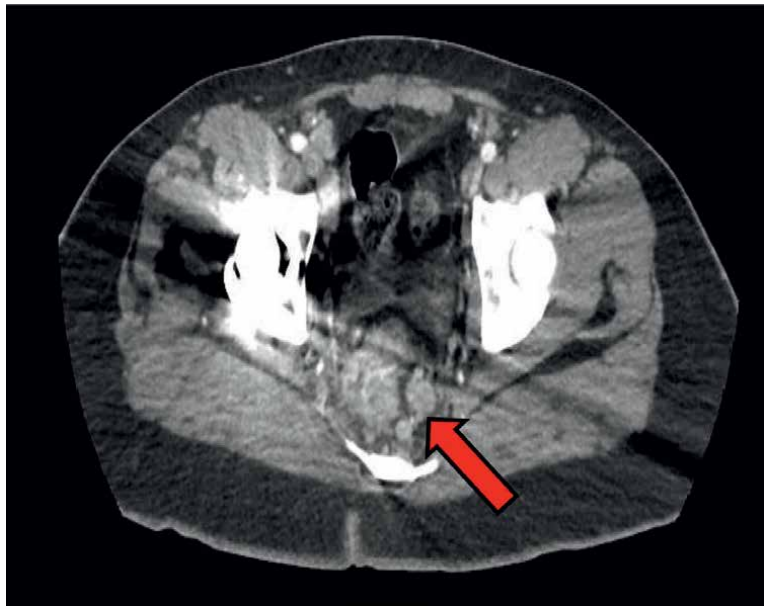

Figure 2.
Cross-sectional CT imaging of Mpox patient indicating rectal wall thickening (red arrow) [22].

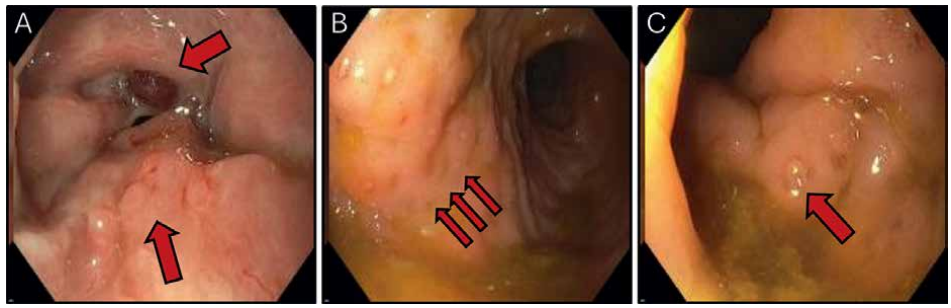

Figure 3.
Endoscopic findings in a patient diagnosed with Mpox. A. Arrows pointing to a rectal ulcer. B. Micro-abscesses seen in the rectum and anus. C. Rectal polyp [22].

Most of the published findings on endoscopy are from the lower GI tract (flexible sigmoidoscopy or colonoscopy), likely because patients present with symptoms of proctitis, rectal pain, rectal bleeding, or tenesmus. Endoscopy findings are notable for proctitis, a unique presentation in the most recent outbreak, as well as mucosal ulceration and necrosis. Upper endoscopy revealed the presence of ulcers. If biopsies are obtained, they should be stained with antibodies specific for Mpox to improve diagnostic accuracy.

7. Management of Mpox

Treating patients with Mpox involves early recognition of the disease, institution of appropriate precautions, consultation with an infectious disease team, and the application of medical therapy.

7.1 Isolation precautions during hospitalization

Once a patient has been identified as having Mpox infection, they should be placed in an isolated room with their own bathroom. For any procedures involving the airway or endoscopy, it is recommended that they be performed in an airborne infection isolation room. Healthcare personnel should wear personal protective equipment (PPE), which includes gowns, gloves, eye protection, and respirators.

If patients are suspected to have Mpox infection, they should be placed in isolation until the infection is ruled out. Patients with confirmed infection should be placed in isolation until all lesions have crusted, crusts separated, and a fresh layer of healthy skin has formed underneath.

If a patient is exposed, they will need a 21-day monitoring period, which includes a daily skin exam. If patients present with other symptoms without a rash, the CDC recommends empirical isolation precautions for 5 days, which can extend beyond the original 21-day period [28].

7.2 Isolation precautions during hospitalization

Gastroenterologists can be exposed to patients with Mpox during clinical interactions and endoscopy. For clinical examinations that involve examination of the anorectal area, precautions should be taken, which should include ensuring that patients are placed in isolated rooms and PPE is donned appropriately. Proper sterilization of the endoscopy suite and precaution for reusing it to ensure adequate clearance of the virus, as well as sterilization of the equipment according to CDC guidelines, is recommended. As the virus can exist for days to weeks at room temperature outside the body, this must be taken into account before clearing a room prior to repeated use. Appropriate methods for cleaning exposed rooms include chemical disinfectants and ultraviolet (UV) irradiation [29].

7.3 Vaccines for prevention of Mpox

Vaccination is available for pre- and post-exposure prophylaxis. The vaccines available are the Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) vaccine and the ACAM2000 vaccine [30]. Given the self-limiting nature of infection in immunocompetent individuals, pre-exposure prophylaxis vaccination is reserved for persons with high behavioral or occupational risk factors. Pre-exposure prophylaxis is usually given with the MVA Vaccine. Two doses are given 4 weeks apart [31]. The MVA Vaccine is used over the ACAM2000 since it is associated with less adverse effects [32]. Vaccination can help reduce the risk of Mpox and help protect against severe symptoms if infection does develop.

Post-exposure prophylaxis is performed with the MVA Vaccine as well. Post-exposure vaccination is usually performed for high-risk exposures in either the community (sexual contact of mucous membranes or contact of mucous membrane with bodily fluids of individual with Mpox) or health care (contact of mucous membrane with bodily fluids of individual with Mpox) setting [33]. Post-exposure prophylaxis can be performed for intermediate-risk exposures on a case-by-case basis.

7.4 Treatment of Mpox

Tecovirimat (TPOXX, ST-246) is the first-line therapeutic option for Mpox treatment. Other additional therapeutics are Brincidofovir (CMX001 or Tembexa) and

Vaccinia Immune Globulin (VIGIV) and Cidofovir (Vistide) for patients who need additions/alternative treatments [34].

Once a diagnosis of monkeypox is established, treatment should be tailored to the patient's immunocompetence and presenting symptoms. If a patient is immunocompetent, the infection is relatively mild and usually clears without significant medical intervention. If pain is present in these patients, supportive care with pain control directed at the area causing pain should be performed, in addition to pain management with oral NSAIDS and/or acetaminophen. Stool softeners and lidocaine can be considered in immunocompetent patients with anorectal involvement [35].

For patients who are immunosuppressed, at risk for disseminated infection, pregnant, under the age of 18, and/or subject to a severe monkeypox infection, antiviral therapy is initiated. Tecovirimat, an orthopoxvirus protein inhibitor, is the antiviral agent of choice. Although Tecovirimat is well-tolerated and has been shown to have survival benefits in animal models, studies are still being performed to assess its full efficacy in humans with active disease. Treatment usually lasts for 14 days but can be longer [34, 36, 37].

To assist with the assessment of the patient and initiation of appropriate antiviral treatment, infectious disease team should be consulted early in the patient's course. They should also work closely with gastroenterology physicians for diagnosis, treatment, and follow-up. Once treated, patients should be examined for any signs or symptoms of recurrent infection for at least 21 days. If no changes occur during that period, the patient may return to all activities of daily living without restriction. If patients develop worsening symptoms, they are to immediately contact the health department for the next steps. Patients should also frequently follow-up with gastroenterology, infectious diseases, and primary care providers.

8. Conclusion

The recent global outbreak of Mpox after the COVID-19 outbreak highlights the need to equip healthcare providers and patients with the right tools and treatment for prevention, early diagnosis, and treatment of patients infected with Mpox. A low threshold for Mpox should be placed in high-risk patients, particularly those patients who are MSM and HIV-positive. The most common physical exam finding is skin lesions with a characteristic appearance. Systemic symptoms such as fever and malaise are present. As the focus of this chapter is on gastrointestinal presentation of Mpox, there are several published reports on symptoms such as proctitis, rectal pain, and tenesmus. Patients with a history of Mpox should be placed in isolation according to the guidelines published by the CDC or guidelines per the hospital. Laboratory work and imaging, particularly of the abdomen and pelvis, can are useful diagnostic tools. If endoscopy is planned, appropriate staining should be performed to correctly diagnose Mpox. A multidisciplinary team should be engaged, which includes the infectious disease team, gastroenterologist, surgeon, and primary care provider, for post-discharge follow-up. Prophylactic and treatment medications are available for high-risk patients and those infected with Mpox, respectively.

While there are well-published case series and case studies describing various aspects of Mpox infection in various parts of the globe, it is necessary to have a uniform database to store information that can aid healthcare workers to identify the disease early and implementing data-proven treatment strategies. Few studies are available on endoscopy findings and CT/MRI results of patients with Mpox, which

highlights the need to build a database that can aid healthcare providers in terms of what symptoms and findings are expected. A management algorithm for patients with Mpox will also aid in providing quality care by educating providers on evidence-based strategies to ensure better health outcomes.

Acknowledgements

The authors would like to thank all of our patients and acknowledge all of the staff at the Regional One Health Hospital in Memphis, TN as well as the University of Tennessee Health Science Center.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Perspective Chapter: Molecular Diagnostics in Viral Outbreak Surveillance

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Abstract

Understanding and adopting various methods for monitoring viral outbreaks is required for pathogen surveillance. Accurate diagnosis can play a significant role in the safe and effective treatment prescribed. Africa remains burdened with a host of infectious diseases, which challenges healthcare systems and the proper management of infectious diseases. Surveillance systems are implemented in some parts of Africa that have the infrastructure and funding to perform routine testing for pathogen screening. However, not all parts of this continent are equipped and have the necessary tools and support. With travel, tourism and goods exchange, infectious diseases can spread across borders rapidly, posing a threat to global health, emphasising the unified need for efforts to prevent, detect, and act on disease management through improved access to vaccinations and treatments. Effective response to disease outbreaks depends on several elements, including laboratory capacity, skilled health staff, and effective surveillance systems to detect and limit the spread of infectious illnesses rapidly. Traditional molecular methods such as genotyping and polymerase chain reaction (PCR)-based detection systems are now being complemented with tools like next-generation sequencing and clustered regularly interspaced short palindromic repeats (CRISPR). This book chapter aims to summarise the current methods and tools required for viral pathogen surveillance and broadly describes the issue of climate change and its impact on viral outbreaks.

Keywords: surveillance, molecular diagnostics, viruses, outbreaks, pathogen

1. Introduction

Disease outbreaks are driven by infectious pathogens that contribute to large-scale pandemics. Over the past two decades, there have been notable viral outbreaks globally such as SARS-CoV-2, Dengue, Zika, Ebola, Chikungunya, Swine Flu and Oropouche [1]. The cause of the emerging and re-emerging viruses has been due to climate change, urbanisation, increased travel, ageing with decreased immunity and land use change. This has changed the dynamics in certain areas between human and animal interactions [2]. These outbreaks cause severe threats to global health and impact the economy and livelihoods of all affected individuals [1]. Therefore, one of the main

lessons learnt from the SARS-CoV-2 pandemic was the importance of being prepared in terms of surveillance strategies, lab diagnostic tests, upskilling of personnel and the ability to translate the results to relevant health authorities rapidly.

Over 7 million deaths were attributed to the COVID-19 pandemic, driven by the re-emergence of a type of severe acute respiratory coronavirus (SARS-CoV) [3]. Viral outbreaks threaten global public health, often leading to widespread illness, death, and economic disruption. Rapidly identifying and tracking emerging viruses are essential in mitigating the impact of such outbreaks. Molecular diagnostics, particularly techniques that analyse viral nucleic acids (DNA or RNA), have revolutionised the detection, surveillance, and management of viral outbreaks. These techniques offer unparalleled sensitivity, specificity, and speed, enabling public health authorities to respond effectively to emerging infectious diseases. The use of molecular tools, such as polymerase chain reaction (PCR), Next-Generation Sequencing (NGS), and clustered regularly interspaced short palindromic repeats (CRISPR)-based technologies, has become critical for not only diagnosing infections but also tracing viral evolution, detecting mutations, and understanding viral transmission dynamics [4].

Molecular diagnostics allows for the identification of pathogens in asymptomatic individuals and is helpful for indicating previous exposure when assessing a specific virus, aiding scientists and healthcare authorities in controlling transmission [5]. Clinical and public health laboratories play a significant role in the diagnosis and treatment of microbial pathogens, which is important for surveillance and the response to outbreaks of viral pathogens [6]. During the COVID-19 pandemic, PCR-based diagnostics enabled the detection of SARS-CoV-2 around the globe, allowing for early intervention and widespread testing to curb the virus' spread. In addition, molecular surveillance tools report on valuable data such as viral genetic mutations, facilitating the monitoring of viral evolution and the emergence of variants that may evade immunity or become more transmissible [7]. Since the COVID-19 pandemic, next-generation sequencing has emerged as a crucial tool for genomic surveillance. Its reduced cost has further aided ongoing efforts to enhance the monitoring of infectious pathogens in both clinical and environmental samples, although in low-resource settings, costs can be a challenge [8].

The integration of molecular diagnostics into outbreak surveillance systems is not without challenges. These include the high cost of advanced technologies, the need for specialised infrastructure, and issues surrounding the availability of testing and trained personnel in resource-limited settings [9]. Despite these challenges, the potential of molecular diagnostics to transform outbreak surveillance, offering real-time data and guiding public health decisions, remains immense. This book chapter explores the critical role of molecular diagnostics in viral outbreak surveillance, examining the key technologies, their applications in viral surveillance, and the ongoing efforts to overcome challenges associated with their deployment.

2. Molecular diagnostic techniques

2.1 Overview

Disease diagnostics plays a significant role in the public and healthcare sector, with agility, speed and flexibility being the three targets when selecting a method for accurate and effective diagnosis. Molecular diagnostics helps in the management of these diseases by detecting the genetic material of pathogens in clinical samples.

Earlier techniques such as culture- and PCR-based molecular methods have been the standard procedure for viral outbreak surveillance and pathogen identification [10]. However, some of these methods result in longer turnaround times, quality assurance issues, and accurate decisions [11]. However, recent advancements in next-generation sequencing (NGS), metagenomics, and CRISPR-based diagnostics have revolutionised the field, offering more sensitive, rapid, and comprehensive methods for monitoring and controlling viral outbreaks [12]. These newer methods have become relatively affordable and sustainable in laboratory implementation for surveillance purposes and are slowly becoming the preferred method globally.

2.2 Significance

Africa is undeniably burdened with the highest rate of infectious diseases, coupled with the weakest public health infrastructure globally. Moreover, establishing effective public health systems may take years or even decades. Emerging infectious diseases must be prioritised. Challenges include integrating surveillance with epidemic preparedness and response efforts for priority diseases. This task is challenging due to the limited infrastructure and support for surveillance, research, and training on emerging diseases in Africa. Implementing laboratory-based surveillance and conducting focused research surveys to identify common infection sources in various communities could provide a unified approach to tackling this vast challenge. The most crucial step towards reducing Africa's infectious disease burden is a substantial increase in the number of qualified personnel, including physicians and scientists [13]. Emerging infectious diseases in sub-Saharan Africa include cholera, meningitis, Ebola, measles, yellow fever, monkeypox, Zika, Rift Valley fever, and COVID-19. Several factors contribute to their rise, such as microorganisms adapting to climate and environmental changes, shifting ecosystems, and increased human vulnerability due to immunosuppression, malnutrition, and inadequate immunisation [14]. Enabling low-resource countries to be prepared for emerging and re-emerging viral outbreaks will help manage the constraints faced by the healthcare systems and government [11].

2.3 Established techniques

2.3.1 PCR-based methods

Polymerase chain reaction (PCR) has been one of the primary methods used to identify viruses [15]. PCR was reported as the most common method used for diagnosing epidemic-prone diseases, followed by ELISA in a survey conducted by the Africa CDC and the European Centre for Disease Prevention and Control (ECDC) on all 55 African Union states [16]. For this method, RNA is extracted and reverse-transcribed to cDNA in the case of RNA viruses, followed by specific primers that span and amplify a region of interest. One such example commonly used during the COVID-19 pandemic was the use of tiling primers, which are primers designed to generate short amplicons that overlap each other and cover the whole region or genome of interest [17]. However, a limiting factor in this approach was the need to regularly update the primers to ensure its sensitivity to current viral variants [18]. Quantitative real-time polymerase chain reaction (qRT-PCR) enables the quantitative detection of amplified genetic material using a fluorescent marker, making it the gold standard for monitoring infection progression and therapy response [19]. Automation

and high-throughput advancements in data analysis have further solidified qRT-PCR's dominance. SYBR green and TaqMan assays are examples of qPCR technologies that can help identify and quantify amplification products [12]. Older methods like transcription-mediated amplification and loop-mediated isothermal amplification have gained popularity [20]. Digital PCR (dPCR) is another PCR method that offers absolute quantification without external calibration curves, making it useful for drug-resistant variants and patient management [21]. Although PCR has been widely used to detect the presence and absence of pathogens in a sample, prior knowledge of the potential pathogens is required, and this may not be an ideal approach for outbreaks caused by novel or unknown pathogens [22].

2.3.2 Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) assays were established for the Zika virus, H5N1 avian influenza virus [23–25] and SARS-CoV-2. Loop-mediated isothermal amplification (LAMP) has been widely used for the detection of pathogens. It uses three primer pairs for selective binding and a DNA polymerase with strong strand displacement capabilities [20]. LAMP has advantages over PCR whereby it eliminates the need for multiple temperature cycles and long reaction times, which can lead to PCR errors [20]. This approach aims to generate a large number of DNA amplification products with an alternating, repetitive structure and compatible sequence [12]. Previously, its use was challenged by non-specific binding due to the formation of primer dimers. Furthermore, the use of multiple primers increased the risk of primer-primer hybridisations, resulting in false-positive results [26]. More recently, this approach has been modified to accurately identify dengue virus, Human Immunodeficiency Virus (HIV)-1, and Zika virus (ZIKV) in clinical samples [27].

2.3.3 Culture-based methods

Although some researchers may view the traditional cultural method of viral discovery as outdated, costly, and impractical, it remains in use for several key reasons. Growing a virus in culture provides an enriched template for molecular analysis and serves as an inoculum for animal models, serological tests, and neutralisation assays, which support research into the aetiology of infectious diseases, surveillance and mitigation of viral outbreaks. Avenues such as vaccine testing and drug evaluations are some areas that benefit from stock cultures. However, identifying appropriate cell lines may be challenging and innate immune responses can obstruct these processes, but they can be mitigated using antibodies or molecules that target specific pathways [28]. The culture method was previously thought to be the gold standard for identifying microorganisms. However, molecular methods display the advantage of quickly providing results and revealing unknown microorganisms undetected by culture [29].

2.3.4 Microarrays

Microarrays are a technology designed to conduct complex, parallel tests based on ligand-binding, such as oligonucleotides, which are positioned on a solid support with high packing density to identify a complex mixture of target sequences. For biological applications, the ligands on the array can include DNA, RNA,

proteins, polysaccharides, lipids, small organic compounds, or even whole cells. DNA microarrays are the most widely known and popular among these various ligands [30]. This technology is applied in genotyping, chromatin immunoprecipitation, gene expression and transcription factor binding assays [31]. DNA microarray technology significantly influences how molecular biologists view genes and is driving a shift towards a post-genomic era, focusing on structural and functional genome analysis [30]. A study conducted by Yao et al. [32] showed how affordable, sensitive and specific a multi-pathogen microarray was for the detection of Ebola virus and haemorrhagic fever and confirmed its use in the prevention, treatment and surveillance of Ebola.

2.4 Emerging techniques

2.4.1 Next-generation sequencing

Significant developments in sequencing technologies have been made in the past 10 years; these systems provide high-throughput sequencing features and fast assembly of genomes from challenging metagenomic samples in line with an associated cost reduction [33]. With the parallel sequencing of millions to billions of DNA fragments made possible by modern NGS platforms such as Illumina, Pacific Biosciences, and Oxford Nanopore, the field of genomics has been revolutionised [34]. Next-generation sequencing during outbreaks can be used to detect new viral pathogens, study interactions between the host and virus and perform metagenomic analysis of the human virome [15].

2.4.2 Metagenomics

Molecular assays are increasingly used in clinical diagnostics due to speed, sensitivity, and accuracy improvements. The need to create and synthesise PCR primers and probes is eliminated by metagenomics [35]. This saves time, which is important when viral illness outbreaks occur. One of the applications of next-generation sequencing is metagenomics. Metagenomics could be targeted or untargeted. The untargeted approach is better known as shotgun sequencing and allows for sequencing every possible pathogen in a sample [36]. Early viral sequencing from an outbreak aims to identify and locate the reservoir host while characterising the responsible virus [22]. Metagenomics allows for the identification of any pathogen present in a sample, and the clinical application was demonstrated by the use of metagenomics to identify SARS-CoV-2 within the first 4 weeks of the first case [37]. This approach has its challenges mainly due to the high proportion of host DNA in comparison to the pathogen of interest, therefore requiring careful nucleic acid extraction procedures and removal of host DNA [36].

Furthermore, deep sequencing is required to ensure sufficient reads are acquired following removal of background noise and host sequences, and this could increase the costs of sequencing considerably. Complicated sample preparation methods greatly reduce repeatability due to several procedures and protocol variances, which raises error rates [35]. This is a significant obstacle to the clinical use of metagenomics. Targeted approaches consist of specific primers designed based on conserved regions in bacteria (16 metagenomics) and fungi (ITS metagenomics). Viral metagenomics can be slightly challenging due to the low abundance of viral material compared to host material.

2.4.3 Target enrichment sequencing

Hybridisation probe-based capture sequencing is a targeted method that sequences specific regions of interest using short biotin-labelled oligonucleotide probes that complement and hybridise to the selected viral genome [35]. This approach allows for the detection of low-abundance viruses, and it reduces sequencing costs by focusing on relevant genomic regions [38]. Targeted sequence capture is a validated method for enriching specific nucleic acid sequences, providing an alternative approach for the selective isolation of pathogen-derived nucleic acids in metagenomic samples [36, 39]. Commercial assays such as the Illumina Viral surveillance panel, VircapSeq VERT, IDT xGen Custom Hybridisation Panels and Twist Comprehensive Viral Panel are available for this approach. Next-generation sequencing has proven particularly valuable when standard tests fail to provide a diagnosis. A study by Zhou et al. [40] analysed 121 viral clinical samples using the Luminex xTAG RVP assay and NGS random sequencing. The samples were prescreened for cytopathic effects and initially tested negative for influenza. This study compared the Luminex xTAG Respiratory Viral Panel FAST test and Roche 454 GS FLX Titanium pyrosequencing to identify pathogens not typically monitored in hospitals. NGS confirmed all viruses detected by Luminex and identified additional respiratory viruses, including Dengue, which is not typically a respiratory pathogen. Bacteria such as *Clostridium difficile* and *Acinetobacter baumannii* were found, suggesting nonviral infections could also be detected. While viral culture enriched viral content for NGS, making it more effective in detecting viruses, it may not always be feasible due to cost and contamination risks. Combining viral culture, molecular assays, and deep sequencing has enhanced our understanding of the prevalence of viruses, bacteria, and other microbes in clinical settings. The study also revealed a high prevalence of enteroviruses, particularly EV-B, in respiratory infections. It also produced genome sequences for enteroviruses in Thailand, contributing to knowledge about virus diversity [40].

Due to insufficient data on its relative efficacy, hybridisation probe-based capture sequencing has not acquired much traction yet, and its price per sample is still high [35]. Furthermore, the viral genomes are unlikely to undergo enrichment if they deviate from probe sequences by more than 40% [35]. Therefore, approaches whereby the cDNA synthesis and PCR using degenerate primers may work better, although the output may contain more "noise" data that can be eliminated during processing [39, 41].

2.4.4 Clustered regularly interspaced short palindromic repeats (CRISPR)-based methods

First identified as an immune protection system in prokaryotes, the CRISPR system has recently gained widespread attraction for its potential in gene regulation and editing and has thus proved to be a competitive technology in disease diagnostics [42]. CRISPR consists of a Cas protein and a guide RNA (gRNA) which directs Cas to the target site [43]. Researchers can target any gene of interest by changing the gRNA sequence to identify a specific site of interest when configuring the Cas protein to target that specific sequence. This system was recently used to diagnose and treat diseases [44, 45]. Initially used for developing anti-virals, the CRISPR-Cas system later became a useful application in gene-editing and gene-detection ability which revolutionised the diagnostics field. Pathogens in clinical samples can accurately and rapidly be identified with CRISPR point-of-care diagnostics and have also been tested for the

treatment of HIV [46]. COVID-19 has proved that newer infectious disease detection, prevention and management models were required for early diagnosis. Applications such as the CRISPR-Cas systems offer advancements in molecular diagnosis and treatment which can be easily applied and have shorter turnaround times. Viral diseases such as HIV, Tuberculosis, Dengue, Hepatitis B and Zika have successfully been diagnosed and treated with the CRISPR-Cas system. Essentially, this system has been documented to offer great potential for diagnosing disease, detecting RNA viruses, and identifying certain bacteria [47]. With CRISPR diagnostics specific viral sequences are targeted, ensuring high sensitivity and specificity, thereby reducing false positives or negatives commonly seen with traditional methods like PCR [48]. Its accessibility function enables it to be used in remote or resource-poor settings, empowering viral outbreak surveillance in these areas. This is especially important in controlling outbreaks of emerging infectious diseases, and in low-resource settings [49]. Because CRISPR diagnostics target specific viral sequences, they have excellent sensitivity and specificity, which helps reduce false positives or negatives frequently observed with conventional techniques like PCR [50]. The portability of CRISPR allows it to be used in remote or resource-poor settings, enabling local health authorities to monitor and respond to viral threats without the need for expensive and complicated laboratory infrastructure [51]. Additionally, CRISPR can be used for surveillance in areas with limited access to advanced lab infrastructure.

3. Applications in virus/infectious disease detection

Genotyping is a method used to identify genetic variants within individuals and has been a cost-effective means for obtaining genetic information in many individuals [52]. Genotyping data can support healthcare-associated transmission of SARS-CoV-2, for example, fast-track mitigation, management and prevention strategies [53]. Early identification of the organisms and genotypes causing a specific outbreak can quickly improve viral monitoring efforts [54]. By improving outbreak detection and investigation, tracking transmission pathways and networks, monitoring genetic variations affecting pathogenicity, diagnostics, treatments, and vaccines, and assessing the effectiveness of policies and interventions, pathogen genomics can transform public health surveillance completely [55]. Qiu and colleagues [56] developed a fast genotyping method for point-of-care diagnostics using a digital genotyping model and a one-step fluorescent lateral flow immunoassay (LFIA) strip coated with genotype-specific monoclonal antibodies (mAbs). Simple hepatitis B virus (HBV) genotyping results can be acquired in 20 minutes at a very low cost using the recently established technology by Qiu and colleagues, which uses a digital classifier and a simple one-step fluorescent LFIA strip. Because of the streamlined signal detection and analysis techniques and the simplicity to display test findings, the classifier instrument was specifically built using parts that cost less than $100 for POC diagnostics.

4. Genome characterisation and identification

Infections caused by viruses are the most common cause of human diseases [57]. Human Immunodeficiency Virus (HIV) and Hepatitis continue to cause deaths worldwide including re-emerging viruses such as influenza A (H5N1) and Swine influenza

(H1N1), SARS-CoV, Zika virus (ZIKV), and Ebola virus and monkeypox (Mpox) [58, 59]. Although these infectious diseases are detectable worldwide, Africa remains the most affected. Therefore, it is becoming more important for the continent to strengthen its internal capacity to identify, track, and manage the spread of these viral pathogens.

5. Rapid detection systems

Speed and accuracy are essential factors for diagnosing and managing infectious diseases during viral outbreaks. Using molecular techniques such as PCR, NGS, and CRISPR helps public health officials make quick decisions to stop the spread of illnesses and prevent pandemics. Importantly, these molecular diagnostic techniques are sensitive in identifying the existing pathogen and characterising its genetic profile, which is essential for diagnosing diseases in the early phases of an outbreak. This information enables the identification of viral genetic material necessary for detecting new or emerging viruses that conventional diagnostic methods may miss [10]. Rapid molecular detection systems are essential for viral outbreak monitoring as they provide early alerts and diagnosis, particularly in asymptomatic individuals. Therefore, molecular diagnostic tests help detect viruses and mutations, thereby aiding in tracking the dissemination of viruses and recognising new variants. Advancements in these diagnostic tests can also allow for portability, enabling on-site testing in remote locations and accelerating the decision-making process [60]. Moreover, molecular data may be incorporated into worldwide surveillance systems, facilitating a unified reaction and allowing for more precise public health measures.

6. Viral load quantification

Viral load is the amount of virus in an infected person's body and is typically measured in samples like blood or respiratory fluids. It is required for tracking viral outbreak surveillance because it provides data on disease severity, patterns of transmission, and public health responses [61]. A high viral load often correlates with higher infectivity, implying that these individuals are more likely to spread the virus to others, especially during the early stages of infection. By examining viral loads in a population through testing and sequencing, health authorities can locate regions with active transmission, assess the virus's reproductive number (R0), and detect potential outbreaks before they escalate. During the COVID-19 pandemic, research indicated that SARS-CoV-2-positive individuals with high viral loads were more infectious and contributed to spreading the virus during contact tracing and isolation measures [62]. Furthermore, tracking viral load trends over time could shed light on changes in virulence, as lower loads suggest a reduced risk of spread [63, 64]. On a broader scale, wastewater monitoring uses viral load evaluations to detect the presence of a virus in a population, including among those without symptoms, serving as an early warning system for potential outbreaks. As a result, viral load data enhances surveillance by gauging the virus's spread and enabling swift, targeted responses [65]. Measuring viral load within virus-infected individuals is an important step for viral outbreak surveillance, specifically for infectious diseases such as HIV, influenza, COVID-19 and hepatitis. Evaluating a patient's viral load provides important information about prognosis and predictions related to viral outbreaks, transmission trends, and the effectiveness of potential treatments [66].

7. Epidemiological surveillance and monitoring

Epidemiological surveillance and monitoring are crucial for disease management and being ready for a pandemic. Emerging and re-emerging pathogens continue to burden the already pressurised healthcare systems, especially in low and middle-income countries, by challenging pharmaceutical industries and overwhelming medical care. Circulating viral strains may harbour genetic variation due to mutations that arise over time. These genetic variations could compromise the effectiveness of current diagnostic tests [67]. Poor documentation of surveillance data makes it challenging to implement timely and effective interventions [68]. Despite the significance of this data, endemic countries still lack adequate surveillance for high-priority diseases [69, 70]. Such challenges, especially in low and middle-income countries, prohibit the tracking and spread of pathogens in communities, identifying populations at high risk, and determining the impact at a local and global level. Data gaps in this context delay response efforts and the potential for the development of accurate predictive models and early warning systems. Improving global surveillance systems and ensuring that emerging pathogens are closely monitored is essential for improving pandemic preparedness [8]. Early detection and monitoring of disease trends aid in surveillance systems, reducing the impact of transmission and strain on healthcare management. With advancements in data acquisition and analysis, including the integration of machine learning and real-time reporting, public health authorities are enabled to better manage viral outbreaks, minimising their spread and safeguarding public health [71].

Existing surveillance systems should be leveraged and adapted for the surveillance of pathogens. The COVID-19 pandemic paved the way for viral genomic surveillance, showing its importance for public health. Hill et al. [72] prompted a call for action for global virus surveillance networks to provide data on viral evolution and lineage transmission during and in between outbreaks. The main aim of this endeavour is to provide a streamlined workflow for labs to rapidly sequence different viruses, analyse the data and respond to emerging viruses [72]. Using existing infrastructure for surveillance of new viral pathogens can be challenging, particularly when changes to lab protocols are needed. Most SARS-CoV-2 sequencing labs follow a standardised workflow that includes sample selection, viral amplification, library preparation, sequencing and data analysis. Adapting an existing pipeline for new viruses may require modifications, especially with sample preparation and bioinformatics processing. A typical example is SARS-CoV-2, which relies on a targeted, multiplexed tiling PCR-based approach, whereas sequencing a virus like monkeypox traditionally requires metagenomic sequencing [73]. For many viruses, the rest of the library preparation and sequencing workflow can remain the same by simply using target-specific PCR primer sets. This approach allows researchers to repurpose their existing expertise and infrastructure for surveillance of various viral pathogens [72].

8. Challenges and limitations of molecular diagnostics

Half of the world lacks access to essential healthcare services placing a significant strain on those who cannot afford these services. The World Health Organisation has encouraged policymakers and authorities from developing nations to incorporate eHealth to improve accessibility to healthcare, and quality of services and to achieve the United Nations Sustainable Development Goal 3 to "access to quality essential

health-care services" [74]. However, the study by Asah and Kaasbøll made a few remarks on the difficulty of integrating eHealth in low- and middle-income countries (LMICs) as well as developing countries. These include limited funding, poor infrastructure, and poor governance which could be improved with computational systems, in-service training, and advancing academic modules. To solidify this implementation, it would be beneficial to have relevant eHealth policies and guidelines supported by committed leaders, thereby building global healthcare capacity [74]. Molecular diagnostics can be more effectively used in healthcare when combined with eHealth services to advance prevention, management and preparedness strategies in an outbreak.

Sequencing was mostly used as a research tool or not at all in LMICs prior to SARS-CoV-2 [8]. Implementing pathogen genomics remains difficult in many low-resource settings due to a lack of qualified staff, poor laboratory infrastructure, and a restricted ability to use genomic data in public health response [75]. It is crucial to keep in mind that not all African nations may find sequencing feasible, and diagnosing and treating infectious diseases may present significant challenges. Pathogen sequencing in Africa has advanced the diagnostic landscape for accurately detecting, identifying and monitoring disease outbreaks from emerging and re-emerging pathogens and facilitating data exchange. Since the COVID-19 pandemic, African countries have established capacity for molecular testing and genomic surveillance of SARS-CoV-2 variants instead of exporting samples. Decreased sequencing costs have resulted in massive volumes of genomic sequencing data around the world, bringing upon a high demand for skilled bioinformaticians to analyse and interpret data [76].

Gaps in molecular diagnostics were identified during the COVID-19 pandemic, with infections in sub-Saharan Africa going undetected for months because of low-resource healthcare systems [77]. Integrating pathogen genomics into public health initiatives in LMICs requires conquering hurdles, which comprise offering training programmes, improving laboratory capacity and increasing the availability of affordable sequencing platforms. Until these challenges are addressed, the full potential of molecular diagnostics in controlling infectious diseases and improving patient care may remain out of reach for many in these regions. Another factor is the availability of computational resources and bioinformaticians to process and analyse genomic sequencing data [76].

Aside from COVID-19, Ebola and Yellow fever were detected weeks earlier with the help of molecular diagnostics compared to standard methods using on-the-spot PCR testing [11]. Combining culture-based methods and whole genome sequencing enabled the identification of atypical pathogens and antimicrobial resistance profiles in Gambia [78]. The global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022–2032 was designed by the World Health Organisation (WHO) to support national genomic surveillance efforts in pathogen detection. This strategy also highlighted the need for efficiently collecting and disseminating genomic and antimicrobial resistance (AMR) data to aid decision-making on epidemic response.

A review by Chidzwondo and Mutapi [79] discusses the burden of disease, co-infections and the challenges in managing specific diseases in Africa, these include Ebola, Plasmodium species and cholera. Debilitated health systems and insufficient response tools worsen the impact of these diseases. Hence, it is important that diagnostics for infectious diseases occur quickly, infected individuals are isolated, and the right treatment is prescribed to contain disease outbreaks by actively working together to develop rapid point-of-care diagnostic tools for effectively monitoring, managing and mediating actionable changes for sustainable healthcare in Africa [79].

To strengthen outbreak management and emergency response, it is imperative that the correct disease is diagnosed and pathogen identified which will assist with the applicable treatment in containing the spread of viral infections. The fear of the unknown was emphasized in relation to understanding and responding to public health emergencies where the situation at hand is unknown [80]. Even though the United States of America (USA) and Mexico had a pandemic influenza plan in place from 2004 to 2006, it was designed for an outbreak that would occur far away rather than anticipating that it would occur nearby. Coherent procedures should also be in place for determining when it is appropriate to proceed to the next level of response, putting clinical procedures and operations into place, and making decisions in the middle of the course. Models should include flexibility and adaptability to assist with the action plan for individual needs, even though many emergency plans can be narrative and administratively focused [80].

The exponential era of data-driven research generates significant digital data in different sectors such as research, industry, policymakers, health officials and data scientists. Governments, international development agencies, and multilateral organisations are requesting digital data to be considered a public good and made publicly available. A 2024 study by Cengiz and colleagues [81] explored the advantages and challenges of sharing health-related research data across borders. The study found that past exploitation and unequal resource allocation in African institutions lack data-sharing agreements, and because of this, African researchers require more robust government structures that place a high priority on accountability, openness, and fair benefit distribution to protect their data, advance their skills, and facilitate efficient sharing procedures. Since the primary owners have no control over users who access, reuse their data, or recover costs related to data collection, preparation, and unauthorised use, making data openly and freely available has not gained traction in the research community. For private research activities, the disparity in rewards and risks has led to data exclusion and/or protection through a variety of mechanisms, including licencing, legal protection, and geographic and climatic restrictions. It also discourages open data sharing and restricts data release. In theory, if there is a chance that researchers would profit from their investment, they will be encouraged to make their data publicly accessible. Since owners have not been able to control access, reuse their data, or recover costs related to data collection, preparation, and unauthorised use, making data openly and freely available has not gained traction in the research community [82].

A wide variety of methods are available for rapidly detecting epidemic signals through both standard and syndromic surveillance. These techniques can generally be categorised based on temporal clusters, with several outbreak detection algorithms falling under each category. However, a key challenge is that health and public health professionals often struggle to understand all the methods available for continuous data monitoring within surveillance systems and to determine when to implement them. Furthermore, with regards to NGS, establishing validation thresholds and creating a standard strategy for NGS is important and must be resolved before implementation in clinical practice [35]. Another limitation is that the algorithms used in viral surveillance are tested with real data, while only a few studies have used fully simulated, reproducible datasets. In contrast, real-world datasets are rarely free, limiting the ability to conduct robust, authentic, and meaningful research worldwide. As a result, comparing algorithms becomes difficult due to the varying results and assessments across different studies [83].

Genomic sequencing helps track how viruses evolve and spread, but to understand its impact on public health the genomic data needs to be combined with data

on patient demographics, epidemiology and clinical outcomes. These are necessary for identifying links between specific viral strains and factors such as patient risk profiles, disease severity and transmission rates. By linking genomic data with details such as demographics, clinical outcomes and epidemiological trends, scientists can uncover which viral variants are more dangerous, spread more easily, evade immunity, or respond differently to treatments [84]. Therefore, accessibility and sharing of research data across and within borders is necessary.

Finally, having robust viral diagnostic methods, analysis pipelines and sample metadata during an outbreak is ineffective in controlling the spread of infection during an outbreak if the results are not reported to public health authorities in a timely manner [85]. Effective reporting for viral surveillance requires timely and comprehensive data. However, ensuring this requires technical expertise, reliable data-sharing networks, and standardised reporting protocols to ensure that information is shared efficiently between all relevant individuals [84].

9. Future directions

Artificial Intelligence (AI) has emerged as a powerful and transformative tool in public health applications such as rapid identification, diagnosis, and prediction of infectious disease outbreaks. Previously, epidemics were discovered and predicted based on manual data retrieval, statistical models and clinical observations. Solutions for viral disease outbreaks have now been advanced by integrating AI for its potential to provide quicker, scalable and accurate results [86]. Techniques using AI algorithms for discovering infectious diseases are improved and can be detected earlier, even prior to clinical presentation in patients. Machine learning methods enable the analyses of vast amounts of clinical data, such as medical images, patient records and genetic sequences, to uncover fine details and patterns indicative of an outbreak [87, 88]. AI models are being trained with data collected from previous outbreaks to identify, characterise and predict the emergence of new and future pathogens, strains or variations, thereby enabling quicker response and actions for mitigation and management [89, 90]. Hence, it is essential to merge clinical virology with advanced technologies such as artificial intelligence (AI), machine learning (ML), and deep learning (DL) because of their competence in handling extensive datasets, their capability to learn pattern recognition, and their potential to deliver precise and prompt answers.

9.1 Predictive outbreaks

AI's predictive functionality can assist in forecasting the spread of infectious diseases, and simulations from these predictions can uncover how a specific disease or pathogen is likely to spread across communities and populations by using previous outbreak data, demographic information, environmental factors, and travel patterns. Altogether, these models can help predict when and where an outbreak is likely to occur, its potential severity, and the effectiveness of different intervention strategies [91]. An example is using AI algorithms from epidemiological modelling, which incorporate machine learning processes like neural networks, random forests and support vector machines to predict the trajectory of influenza, Ebola, and zika virus [92]. Continuous integration of real-time data in public healthcare records, airports and hospitals can be routinely refined and processed to provide almost immediate

predictions that guide and inform decision-making. Moreover, AI strengthens the monitoring of climate change impacts on driving infectious diseases. Estimating future seasonal epidemics can be improved with machine learning algorithms that can examine data from vector populations such as mosquitoes spreading malaria or dengue, temperature and precipitation records [93].

9.2 Enhancing surveillance systems

Surveillance systems have been one area supported by advancements through the automation of large volumes of data collection and analysis. Previously, health systems used manual reporting and analysis, which required time and has now been improved with AI to automate these processes, essentially detecting early signs of a disease outbreak and predicting the nature of an outbreak from certain locations [94]. During the COVID-19 pandemic, major growth in digital surveillance of infectious diseases has been achieved using computational models. Since then, the migration towards integrating cloud-based data approaches has shifted to develop newer and refined approaches in improving surveillance of infectious diseases for pandemic preparedness [95, 96]. Integrating AI and internet-based models for monitoring viral outbreaks will significantly enhance predictions and preparedness and support public health officials in their decision-making for future viral attacks.

A qualitative study by Buchbinder et al. [97] describes the ethical and practical concerns when preparing and implementing Data-2-Care (D2C) programmes for HIV-positive people. Suggestions made by some of the stakeholders who participated in this study include overseeing data collection, usage and exchange, privacy and protection against hackers, and verifying data accuracy and security. Sharing public health and surveillance data strengthens the global community by preventing or mitigating the severity of infectious diseases and outbreaks. Public health surveillance data, when shared, enhance the capacity for detecting and responding to disease, whilst finding the source of an outbreak and diminishing its impact [98].

In summary, AI will be a revolutionary tool in advancing diagnostic application and improving prediction models for viral outbreaks. With AI advancement, its role in pandemic readiness and outbreak management will continue to develop, offering new insight and guidance for protecting global health. Known for its efficiency in analysing large data sets, uncovering needle-in-a-haystack trends, and enabling real-time predictions, it equips public health systems with unprecedented tools to identify, prevent, and respond to emerging diseases [99]. Apart from disease predictions, AI will also benefit humans in understanding the aetiology of viral diseases and support drug discovery and vaccine development in the space of infectious diseases [100].

10. Conclusion

In addition to having diagnostic tools available for viral outbreak surveillance, careful planning and coordination is necessary. Genomic epidemiology relies on prompt access to clinical samples and data, which requires effective engagement with local communities, public health agencies, clinics, and researchers [22]. Ongoing surveys to identify obstacles to viral diagnostics and sampling selection techniques will yield important data for upcoming monitoring programmes in addition to the guidelines issued by the WHO and other international public health agencies [75].

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Chapter 8

Perspective Chapter: A New Era in Viral Research during the Pandemic – Can Organoids Serve as an Alternative to Animal Models?

Sevda Demir and Fikrettin Sahin

Abstract

The pandemic era has underscored an urgent demand for more human-relevant and ethical models in viral research. Animal models have been the primary method for studying viral infection dynamics, immune responses, and potential treatments for decades. However, their limitations in accurately simulating human-specific responses raise critical questions about the effectiveness of these models in predicting human disease outcomes. Organoids—three-dimensional, stem cell-derived structures replicating human tissues' architecture and functionality—present a groundbreaking alternative. This review examines the transformative potential of organoids to replace animal models in virology, particularly under pandemic conditions that require rapid, precise, and ethically sound approaches. With their ability to closely mimic human tissue environments, organoids enable more accurate analysis of virus-host interactions and more predictive drug screening. Beyond advancing scientific precision, organoids significantly reduce the ethical concerns and logistical challenges associated with animal testing. As the virology field pivots toward these innovative, human-centered models, organoids stand to redefine research approaches, promising a new era in viral research that can accelerate the development of effective treatments and preventive strategies in the face of future pandemics.

Keywords: viral pathogenesis, infectious diseases, organoids, human disease models, 3D cell culture

1. Introduction

Viruses are obligate intracellular pathogens that rely on infecting host cells for their replication. Viral infections can manifest as asymptomatic courses or lead to clinical presentations ranging from mild to severe morbidity and mortality. Zoonotic viruses, in particular, possess the potential to cross-species barriers and cause

epidemics and pandemics in human populations. The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has underscored the substantial impact of zoonotic viruses on global health.

SARS-CoV-2 primarily transmits *via* respiratory droplets expelled through coughing, sneezing, speaking, or breathing by an infected individual. These droplets can be inhaled directly by individuals in close proximity or land on surfaces where they can remain viable for a period of time. Transmission can also occur through airborne particles, particularly in poorly ventilated indoor spaces [1, 2]. Upon entering the respiratory tract, SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of host epithelial cells, primarily in the lungs. Following receptor-mediated endocytosis or membrane fusion, the virus gains entry into the host cell and initiates replication, resulting in cellular damage and a localized inflammatory response [3]. In severe cases, an exaggerated immune response further exacerbates tissue injury, potentially progressing to acute respiratory distress syndrome (ARDS). Beyond the respiratory system, SARS-CoV-2 can also impact other organs, such as the heart, kidneys, and brain. The virus induces systemic inflammation, promotes thrombogenesis, and contributes to multi-organ failure (**Figure 1**) [4].

Understanding human diseases and developing effective treatments remain critical challenges in biomedical research. While animal models have long served as essential tools for elucidating disease mechanisms and evaluating potential therapeutic interventions, they often fail to accurately recapitulate human-specific immune responses, viral-host interactions, and the complex pathophysiology of viral diseases. The COVID-19 pandemic starkly highlighted these limitations. Despite their contributions to our understanding of viral pathogenesis, the development of vaccines, and

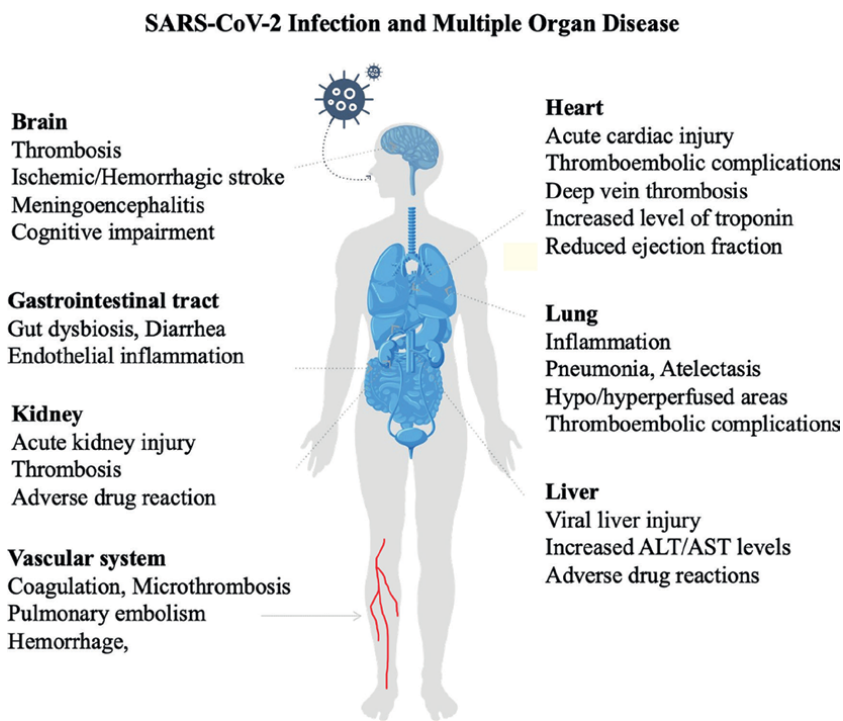

Figure 1.
Potentially affected organs by SARS-CoV-2 infection and their complications.

antiviral therapies, animal models demonstrated significant translational gaps when predicting human responses.

In the pandemic period, widespread lockdowns, and travel restrictions led to significant disruptions in laboratory operations, resulting in reduced access to animal facilities and delays in ongoing studies. These disruptions were compounded by supply chain issues, including shortages of essential reagents and personal protective equipment, further impeding research progress. Moreover, the urgent need to rapidly develop effective treatments and vaccines highlighted the translational shortcomings of animal models, as their predictive validity for human responses was called into question [5, 6]. Another problem encountered during the pandemic is that vaccines and therapies developed for earlier strains often lost efficacy when faced with new variants characterized by altered spike proteins and additional mutations. As a result, the animal models created for these initial strains were unsuitable for accurately representing the virus's changing pathogenesis. This limitation highlighted the need to quickly develop new animal models to capture the updated viral characteristics, further delaying the timely assessment and implementation of effective countermeasures [7, 8]. In viral disease research, working with animal models poses direct risks to laboratory personnel. Handling infected animals increases the potential for zoonotic transmission, as researchers may inadvertently contact infectious agents through bites, scratches, or exposure to contaminated fluids. Moreover, some animals' unpredictable behavior—including aggression or attempts to escape from controlled environments—further elevates the risk of accidental exposure. In addition to scientific and logistical challenges, ethical concerns and the increasingly strict enforcement of regulations pose significant obstacles to the use of animal models.

Given the logistical, scientific, ethical, and safety concerns surrounding traditional animal models, particularly during a global health crisis, there is an increasing impetus to explore alternative approaches that provide faster, more reliable insights into human physiology and disease mechanisms while ensuring enhanced biosafety. Therefore, there is a growing belief that human-based *in vitro* systems and computational models are increasingly being adopted to build more resilient and adaptable research infrastructures. Especially, organoids, three-dimensional constructs derived from human cells that faithfully recapitulate the complex architecture and functionality of native tissues, are a promising alternative to conventional animal models.

In this chapter, the application of organoids in viral research will be evaluated in detail, considering how these three-dimensional structures can overcome the limitations of traditional animal models and whether they provide a more reliable platform for studying viral pathogenesis and evaluating therapeutic interventions.

2. Organoids: A new paradigm in viral research

Three-dimensional cultures, starting with forming simple structures such as spheroids, have been developed using different approaches to generate various human-specific tissues. At the current stage, the integration of complex organoid models, which enable interactions between different cell types with microfluidic systems, has led to the development of organ-on-a-chip platforms, providing results that more closely resemble the clinical condition (**Figure 2**) [9–11]. Among these approaches, organoids have garnered significant attention and are increasingly preferred due to their ability to integrate different cell types into complex structures and be organized according to specific research needs. Organoids are commonly defined

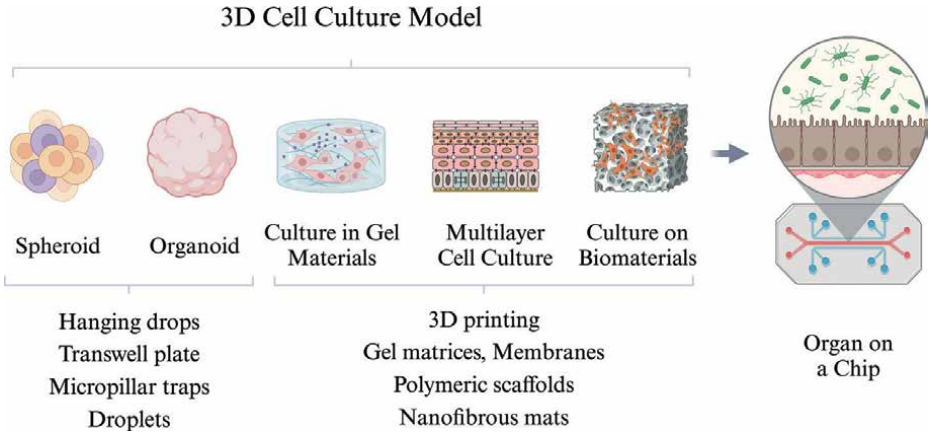

Figure 2.
Different methods used to model human biology.

as complex, multicellular, three-dimensional (3D) *in vitro* culture systems derived from primary tissues or stem cells that retain the key features of their tissue of origin. These miniaturized, organ-like structures exhibit key characteristics of their *in vivo* counterparts, including tissue-specific cell types, spatial organization, and physiological responses [12]. Therefore, organoids have been increasingly utilized in viral research, offering a powerful platform for studying host-pathogen interactions, viral replication, and immune responses in a physiologically relevant context [13].

2.1 Organoid models used in viral research

Organoid models enable researchers to investigate epidemic, pandemic, and non-epidemic viruses in a physiologically relevant context. Understanding the distinctions between these classifications is crucial for comprehending the scope and impact of viral diseases. An epidemic refers to the incidence of an infectious disease surpassing the expected baseline within a community. If this epidemic spreads across international borders, impacting a substantial portion of the global population, it is designated a pandemic, and the virus is called a pandemic virus. Conversely, non-epidemic viruses circulate within populations without causing widespread outbreaks [14].

2.1.1 Non-epidemic viruses and organoids

In non-epidemic viruses, recent studies utilizing human intestinal enteroids have demonstrated distinct infection patterns and immune activation mechanisms for enteroviruses, adenoviruses, and astroviruses. Enteroviruses were shown to infect specific intestinal cell lineages, triggering antiviral signaling in a cell type-dependent manner, thereby revealing the complexity of host immune responses. Adenoviruses exhibited a preferential tropism for goblet cells, highlighting the role of mucus-producing intestinal cells in viral pathogenesis, while interferon signaling was identified as a key factor in restricting viral replication. Similarly, astroviruses displayed multicellular tropism, infecting multiple intestinal cell types and eliciting a diverse innate immune response. These findings underscore the significance of enteroid-based models in virology research, providing an advanced *in vitro* platform

for studying enteric viral infections and developing targeted antiviral therapies with higher translational potential [15–17].

2.1.1.1 Liver organoids

Liver organoids derived from human induced pluripotent stem cells (hiPSCs) have been successfully employed to investigate hepatitis B virus (HBV)–host interactions, supporting viral infection and replication while closely mimicking liver tissue architecture and function. These models provide critical insights into HBV pathogenesis, highlighting viral entry mechanisms, intracellular immune responses, and interferon-stimulated gene activation [18]. Additionally, intrahepatic organoids (ICOs) have been utilized to examine HBV tropism beyond hepatocytes, revealing cholangiocytes as secondary targets and demonstrating inter-host variations in infection susceptibility and immune responses [19]. Beyond HBV research, multicellular liver organoids incorporating hepatocytes, hepatic stellate cells, and Kupffer-like macrophages have been developed to study hepatitis C virus (HCV) infection and its impact on nonalcoholic fatty liver disease (NAFLD) progression. These models effectively replicate key NAFLD features, including lipid accumulation, inflammation, and fibrosis, while also demonstrating that HCV infection exacerbates NAFLD-related pathology, emphasizing the intricate interplay between viral hepatitis and metabolic liver diseases [20]. Collectively, these studies underscore the value of organoid-based platforms in recapitulating human disease pathology, enabling patient-specific investigations, and facilitating drug screening in a scalable and translationally relevant manner. The shared ability of these models to mimic complex tissue-specific interactions and host responses highlights their potential as indispensable tools for advancing virology, toxicology, and regenerative medicine research.

2.1.1.2 Neural organoids

Neural organoids, including cerebral organoids and neurospheroids, have been extensively utilized to investigate neurotropic viruses such as human cytomegalovirus (HCMV), herpes simplex virus 1 (HSV-1), and varicella-zoster virus (VZV). Studies on HCMV infection in brain organoids have revealed its strong tropism for neural progenitor cells, leading to impaired neurogenesis, increased apoptosis, and disrupted cortical layer formation, mimicking congenital microcephaly. Similarly, HSV-1 infection in cerebral organoids demonstrated widespread neuronal cell death, structural disorganization, and a robust neuroinflammatory response, closely resembling viral encephalitis [21–23]. In parallel, VZV-infected neurospheroids exhibited neural tropism and immune evasion, allowing the virus to persist in a latent state, highlighting viral reactivation and neuroimmune modulation mechanisms. These findings underscore the potential of neural organoid models for investigating viral neuropathogenesis, latency, and antiviral therapeutic strategies. Beyond the central nervous system, maternal-fetal interface organoids, including trophoblast and decidua organoids, have been developed to study pregnancy-related viral infections. These models revealed that trophoblast organoids exhibit stronger innate immune activation and resistance to viral infections, while decidua organoids display greater susceptibility, characterized by heightened inflammatory signaling. This differential immune response highlights the complexity of antiviral defenses at the maternal-fetal interface, providing a valuable system for studying virus-induced pregnancy complications [24]. Similarly, ocular organoids, such as conjunctiva organoids, have

been employed to model ocular surface homeostasis and disease. These organoids successfully replicate key epithelial features, including goblet cell differentiation and mucin secretion, making them a useful platform for studying inflammatory ocular conditions such as dry eye disease and conjunctivitis [25]. Together, these studies demonstrate the versatility of organoid models in virology and immunology, providing physiologically relevant systems to explore virus-host interactions, immune modulation, and disease progression across multiple tissue types. Importantly, the shared ability of organoid-based models to recapitulate key structural and functional features of their respective tissues highlights their translational value in therapeutic development, antiviral drug screening, and regenerative medicine.

2.1.2 Pandemic viruses and organoids

Early applications of organoid models in viral research that cause outbreaks focused primarily on studying gastrointestinal and respiratory viruses. Human intestinal organoids, for example, were used to model rotavirus infection, especially for patient-derived rotavirus strains, providing insights into viral replication, antiviral response, and host-pathogen interactions [26]. Similarly, lung organoids have been employed to study influenza virus infection (avian H1N1, H5N6, and H7N9) and respiratory syncytial virus (RSV), enabling researchers to investigate and evaluate cellular tropism, viral pathogenesis, and potential antiviral therapies [26, 27]. These early applications of organoid technology have played a crucial role in studying viruses that cause outbreaks, particularly gastrointestinal and respiratory viruses. However, over time, organoid research has expanded to model different organ systems and become a valuable tool for understanding diseases and developing treatments.

2.1.2.1 Brain organoids

Brain organoids have been widely utilized to study Zika virus (ZIKV) neuropathogenesis, demonstrating that ZIKV exhibits a strong tropism for neural progenitor cells, leading to reduced proliferation, increased apoptosis, and impaired neuronal differentiation. These effects result in smaller, structurally disorganized brain organoids, effectively modeling ZIKV-induced microcephaly. Additionally, infected organoids exhibit upregulated inflammatory responses and dysregulated neurodevelopmental pathways, closely mirroring the neurodevelopmental defects observed in congenital ZIKV infections. These findings highlight the critical role of brain organoids in elucidating ZIKV-induced neural damage, providing a valuable platform for studying viral neuropathogenesis and developing potential therapeutic interventions [28, 29]. To further refine disease modeling, brain-region-specific organoids generated using mini-bioreactors have enabled the study of region-specific vulnerabilities to ZIKV infection. Findings indicate that cortical organoids show the highest levels of viral replication, neuroinflammation, and cellular apoptosis, closely mimicking the cortical thinning observed in microcephaly. In contrast, other brain regions exhibit varying degrees of viral susceptibility and inflammatory responses, emphasizing the region-dependent effects of ZIKV infection on brain development [30]. Beyond disease modeling, brain organoids have been employed for therapeutic screening. Studies using human brain organoids have demonstrated that broad-spectrum antiviral compounds effectively inhibit ZIKV replication, block viral progeny release, and reduce virus-induced neurotoxicity. These treatments have also been shown to

preserve neural progenitor function and restore normal neurodevelopmental processes, highlighting their potential for preventing congenital ZIKV-associated brain damage [31]. Developing self-organized cerebral organoids with human-specific features has further advanced high-throughput antiviral drug screening. Several studies have successfully identified compounds that reduce viral replication, protect neural progenitor cells, and restore neurodevelopmental pathways. Additionally, these models have provided new insights into human-specific cellular responses to ZIKV infection, addressing species-specific differences in viral susceptibility and brain development [32]. These studies highlight the value of brain organoids in modeling congenital viral infections, providing mechanistic insights, supporting comparative viral research, and creating a translational platform for antiviral drug discovery. The capacity of organoid models to replicate species-specific neural responses, evaluate brain-region-specific vulnerabilities, and enable high-throughput drug testing makes them essential tools for understanding viral neuropathogenesis and developing neuroprotective therapies.

Brain organoid-based studies have provided crucial insights into the neurotropism, replication dynamics, and pathogenic effects of SARS-CoV-2, highlighting its potential impact on the central nervous system (CNS). Several investigations utilizing human neural progenitor cells (NPCs) and pluripotent stem cell (hPSC)-derived brain organoids have shown that SARS-CoV-2 efficiently infects neural cells, particularly the choroid plexus epithelium, rather than cortical neurons, resulting in compromised blood-cerebrospinal fluid (CSF) barrier integrity, increased apoptosis, and heightened inflammatory responses [33]. These findings suggest that the choroid plexus may act as a critical entry point for SARS-CoV-2 into the CNS, potentially contributing to the neurological complications observed in COVID-19 patients. Infected brain organoids demonstrated upregulated inflammatory gene expression, structural disorganization, and disrupted neural differentiation, indicating potential neurodevelopmental and neurodegenerative consequences of viral infection [34]. Furthermore, metabolic dysregulation and increased cell death within glioma organoids imply that SARS-CoV-2 may exacerbate glioma progression or alter tumor microenvironments, raising concerns about its impact on neuro-oncology [35]. Therapeutic investigations using sofosbuvir in brain organoids revealed its potential as a neuroprotective antiviral agent, significantly reducing viral replication, preserving neuronal integrity, and mitigating virus-induced inflammation [36]. Collectively, these findings highlight the capability of brain organoid models to replicate key aspects of SARS-CoV-2 neuropathogenesis, providing a physiologically relevant platform for studying CNS infections, evaluating neuroprotective interventions, and understanding virus-induced neuroinflammation and blood-brain barrier disruption.

2.1.2.2 Respiratory organoids

Respiratory organoid-based models, including airway, alveolar, bronchial, and nasal mucosa organoids, have provided crucial insights into viral tropism, replication efficiency, and immune activation, offering physiologically relevant platforms for modeling respiratory viral infections. SARS-CoV-2 has been shown to efficiently infect and replicate within these organoids, leading to epithelial damage, ciliary dysfunction, and dysregulated inflammatory responses, closely resembling *in vivo* lung pathology [37]. In particular, human iPSC-derived alveolar organoids have been used to explore infections with different SARS-CoV-2 variants, revealing variant-specific differences in infectivity, immune evasion, and lung tissue damage, highlighting the

potential impact of viral evolution on disease severity [38]. Additionally, comparative studies on SARS-CoV-2, MERS-CoV, and seasonal coronaviruses using bronchial epithelial organoids have elucidated virus-specific variations in infectivity, cytopathic effects, and innate immune activation, indicating that MERS-CoV induces more severe cellular damage and inflammatory responses compared to SARS-CoV-2 and seasonal coronaviruses [39]. Beyond SARS-CoV-2, strain-specific differences have been identified in influenza A (H5N6/H5N8) viruses, demonstrating efficient replication in human airway organoids, suggesting a high potential for cross-species transmission and human infection [40]. Furthermore, studies employing human nasal mucosa organoids have been instrumental in evaluating early infection dynamics and antiviral drug efficacy, identifying several compounds capable of reducing viral replication and inflammation. These organoid models have also been used to assess the roles of key viral entry mechanisms, such as TMPRSS2 and cathepsins, in facilitating SARS-CoV-2 infection, revealing TMPRSS2 as the dominant pathway in airway epithelial cells [41]. All these findings specified the value of respiratory organoid models in studying viral evolution, predicting pandemic potential, and testing antiviral compounds. The ability of these models to mimic human respiratory physiology, identify strain-specific differences, and evaluate therapeutic interventions highlights their critical role in virology research and drug discovery.

2.1.2.3 Intestinal and kidney organoids

Intestinal and kidney organoids have been instrumental in investigating SARS-CoV-2 enteric and renal infections, particularly in the context of host metabolism, ACE2 expression regulation, and disease severity in high-risk populations. Studies have demonstrated that SARS-CoV-2 efficiently infects intestinal epithelial cells, leading to disrupted barrier integrity, altered immune responses, and metabolic reprogramming, which contribute to the gastrointestinal manifestations observed in COVID-19 patients. Additionally, strain-specific differences have been identified in influenza A (H5N6/H5N8) viruses, demonstrating efficient replication in human intestinal organoids, suggesting a high potential for cross-species transmission and human infection [40, 42]. Comparative analyses have further revealed that different strains of SARS-CoV-2 exhibit variations in replication efficiency and cytopathic effects within the intestinal epithelium, underscoring the need for strain-specific therapeutic approaches. In kidney organoids, SARS-CoV-2 infection has been associated with epithelial damage, oxidative stress, and impaired renal function, effects that are exacerbated under diabetic conditions [43]. Notably, hyperglycemic environments have been shown to upregulate ACE2 expression, facilitating enhanced viral entry and replication, which may explain the increased susceptibility of diabetic patients to severe renal complications. Additionally, the redundancy in viral entry mechanisms within kidney organoids suggests that SARS-CoV-2 can utilize multiple pathways to infect renal cells, indicating that ACE2 inhibition alone may not be sufficient to block infection [44]. These organoid-based models have also been applied to therapeutic screening, identifying potential antiviral compounds that effectively inhibit SARS-CoV-2 replication in intestinal and renal tissues. Moreover, studies have highlighted the role of host metabolic pathways in modulating viral susceptibility, demonstrating that targeting metabolic reprogramming could serve as a viable strategy to mitigate SARS-CoV-2-induced tissue damage [42, 45]. These findings emphasize the critical role of gastrointestinal and renal organoids in studying COVID-19 complications, identifying patient-specific vulnerabilities, and evaluating

potential therapeutic strategies for high-risk populations. The ability of these models to recapitulate disease pathology, assess metabolic influences on viral infection, and serve as platforms for targeted drug discovery highlights their importance in advancing our understanding of SARS-CoV-2 pathogenesis and treatment strategies.

The cardiovascular impacts of SARS-CoV-2 have been studied using human cardiac organoids, providing physiologically relevant models for understanding virus-induced pathology and potential therapeutic strategies. iPSC-derived cardiac organoids have provided key insights into virus-induced myocarditis, cytokine storm-mediated cardiac injury, and contractile dysfunction, revealing that SARS-CoV-2 directly infects cardiomyocytes, leading to metabolic dysregulation, oxidative stress, and inflammatory responses [46]. Infection has been shown to disrupt sarcomere organization, impair mitochondrial function, and activate pro-inflammatory cytokine signaling, contributing to contractile dysfunction and cardiac cell apoptosis [47]. Moreover, cytokine storm conditions, characterized by elevated IL-6 and TNF-α, further exacerbate SARS-CoV-2-induced cardiomyopathy, demonstrating the compounding effects of systemic inflammation on cardiac tissue damage. Importantly, BET inhibitors and other anti-inflammatory compounds have shown potential in mitigating virus-induced cardiac damage, highlighting their therapeutic relevance for COVID-19-associated cardiovascular complications [48]. These studies show that the utility of cardiac organoids in modeling SARS-CoV-2-induced cardiovascular dysfunction, providing critical platforms for understanding disease mechanisms, identifying therapeutic targets, and evaluating potential drug interventions.

2.1.2.4 Vascular immune organoids

Vascularized macrophage-islet organoids and vascular immune organoids have provided critical insights into virus-induced β-cell destruction, immune-driven vascular dysfunction, and inflammatory signaling in SARS-CoV-2 infections, serving as valuable models for studying COVID-19-related metabolic and vascular complications. Investigations have revealed that SARS-CoV-2 infection induces pro-inflammatory macrophage activation, endothelial damage, and disruption of vascular integrity, closely mimicking COVID-19-associated systemic inflammation, coagulopathy, and multi-organ complications [49]. These models have demonstrated that infected macrophage-containing islet organoids secrete high levels of pro-inflammatory cytokines, leading to β-cell pyroptosis, insulin secretion defects, and fibrosis-like structural changes commonly observed in diabetic COVID-19 patients [50]. Furthermore, islet organoid studies have highlighted the role of fibroblast growth factor 7 (FGF7) in upregulating ACE2 expression, increasing β-cell susceptibility to SARS-CoV-2 infection, and exacerbating pancreatic dysfunction in diabetic patients [51]. Notably, hyperglycemic conditions have been shown further to enhance ACE2 expression in kidney and pancreatic organoids, contributing to higher viral loads and increased metabolic dysregulation, emphasizing the heightened risk of severe disease in diabetic individuals [44]. These findings collectively underscore the importance of vascularized and immune-integrated organoid models in studying SARS-CoV-2-mediated metabolic and vascular pathologies. They provide a translational platform for investigating immune-targeted therapies, metabolic interventions, and potential protective strategies against COVID-19-induced pancreatic and vascular dysfunction.

2.1.2.5 Microphysiological systems

Microphysiological Systems (MPS) integrated with multi-organ organoid models have revolutionized virological research, allowing for dynamic interactions between different tissue compartments and creating a more physiologically relevant environment to study systemic viral effects beyond static organoid cultures. Lung-brain MPS models have provided compelling evidence that systemic inflammatory signaling triggered by SARS-CoV-2 leads to blood-brain barrier (BBB) damage, increased neuroinflammation, and endothelial dysfunction, closely mimicking the neurological complications observed in severe COVID-19 cases [52]. These findings suggest that inflammatory cytokines released from infected lung tissue may contribute to secondary neurological damage, highlighting critical pathways for therapeutic intervention. Similarly, bronchial-vascular MPS systems have demonstrated that SARS-CoV-2-induced type-I interferon responses contribute to endothelial injury, increased vascular permeability, and systemic inflammatory damage, resembling COVID-19-associated coagulopathy and vascular dysfunction [53]. These models have been instrumental in identifying molecular pathways involved in virus-induced multi-organ dysfunction, revealing key inflammatory mediators responsible for endothelial damage and immune dysregulation. Additionally, lung-on-a-chip systems combined with airway and alveolar organoids have facilitated high-throughput antiviral drug screening, allowing for real-time monitoring of viral infection dynamics, drug efficacy testing, and precision medicine approaches [54]. These advanced models have been particularly useful for evaluating antiviral therapies targeting SARS-CoV-2 entry mechanisms, immune-modulating treatments, and strategies to prevent lung injury. Collectively, these findings highlight the transformative role of MPS-integrated organoid models in virology research, providing a dynamic and translationally relevant approach to studying multi-organ dysfunction in viral infections. The ability of these systems to recapitulate inter-organ signaling, inflammatory crosstalk, and systemic viral pathogenesis makes them indispensable tools for identifying therapeutic targets and optimizing antiviral strategies in precision medicine.

3. Conclusion

The ongoing advancements in virology research necessitate the development of human-relevant models that accurately mimic viral pathogenesis, immune responses, and therapeutic efficacy. While animal models have historically played a pivotal role in understanding viral infections, their limitations—including species-specific differences, ethical concerns, and translational gaps—highlight the need for alternative systems [5, 6]. Organoids, as three-dimensional, stem cell-derived structures that replicate human tissue functionality, have emerged as transformative tools in virology. These models provide a physiologically relevant microenvironment to study virus-host interactions, overcoming the challenges associated with traditional *in vitro* and *in vivo* approaches [9, 12].

The ability of organoids to closely resemble *in vivo* tissue structures has enabled significant advancements in virology research. They have been extensively used to model a variety of viral infections, including SARS-CoV-2, Zika virus, influenza, respiratory syncytial virus (RSV), and enteric viruses, facilitating the study of viral tropism, replication dynamics, immune modulation, and host-pathogen interactions [28, 33, 40]. For instance, brain organoids have been instrumental in elucidating the

neuropathogenesis of Zika virus and SARS-CoV-2, revealing critical insights into viral entry mechanisms, neural damage, and inflammatory responses [33, 34]. Likewise, respiratory and intestinal organoids have provided valuable platforms for investigating strain-specific differences in SARS-CoV-2 and influenza virus infections, aiding in the identification of antiviral drug targets and therapeutic interventions [40, 42].

Beyond serving as disease models, organoids have proven essential for drug discovery and high-throughput antiviral screening. These platforms have allowed researchers to rapidly assess the efficacy of antiviral compounds, immunomodulatory therapies, and host-directed interventions in physiologically relevant systems [9, 10, 13]. The ability to culture patient-derived organoids has further enhanced personalized medicine approaches, enabling the identification of patient-specific drug responses and the development of precision therapies [13, 42]. Moreover, the integration of microphysiological systems (MPS) with organoids has addressed the challenge of studying multi-organ interactions in viral infections, particularly in the context of systemic inflammation, blood-brain barrier disruption, and cardiovascular complications [52, 53].

Despite these advancements, several challenges remain in the widespread adoption of organoid-based models in virology research. The lack of mature immune components, which limits the ability to fully recapitulate adaptive immune responses, remains a key hurdle [5, 9]. Additionally, standardization of culture conditions, reproducibility across laboratories, and scalability for high-throughput studies are essential areas for improvement. Furthermore, the regulatory acceptance of organoid-based models in clinical and translational research requires further validation to ensure consistency and alignment with existing preclinical models [10, 12, 40].

Nevertheless, as emerging viral threats continue to pose global health challenges, organoid-based models are expected to play an increasingly critical role in pandemic preparedness, precision medicine, and vaccine development [42]. Their ability to bridge the gap between traditional *in vitro* systems and human-relevant disease modeling offers a transformative approach for understanding viral pathogenesis and accelerating the development of novel antiviral strategies. With continued technological advancements in organoid engineering, immune system integration, and multi-organ interactions, organoids can revolutionize virology research and redefine the future of infectious disease modeling and therapeutic development [13, 52, 53].

In summary, organoid-based models are not just an incremental advancement; they signify a transformative approach in virology research. As we navigate the complexities of viral infections and therapeutic development, these models offer unprecedented opportunities for innovation. With ongoing advancements in organoid engineering and a commitment to overcoming existing challenges, the future of infectious disease modeling and therapeutic strategies appears promising. The potential to revolutionize our understanding of viral pathogenesis and enhance pandemic preparedness cannot be overstated, making organoids a cornerstone of modern virology research.

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Edited by Alfonso J. Rodriguez-Morales

In an era marked by the frequent emergence and re-emergence of viral threats, understanding the dynamics of outbreaks has never been more urgent. From SARS and MERS to Ebola, Zika, mpox, influenza, and COVID-19, viral outbreaks continue to challenge health systems, disrupt societies, and expose global vulnerabilities, particularly in low- and middle-income countries. *Current Topics in Viral Outbreaks* provides a critical and up-to-date exploration of the most pressing issues in outbreak science and response. This volume brings multidisciplinary perspectives on viral pathogenesis, transmission dynamics, diagnostics, clinical care, public health interventions, and pandemic preparedness. It also examines the roles of environmental change, urbanization, global travel, and One Health interactions in shaping outbreak patterns. Emphasizing equity, scientific rigor, and cross-sectoral collaboration, this book offers actionable insights for mitigating the impact of viral epidemics and enhancing resilience at local, national, and global levels. A valuable resource for infectious disease specialists, epidemiologists, public health practitioners, and policymakers, this work contributes to the knowledge foundation needed to anticipate, detect, and respond effectively to current and future viral threats.

Alfonso J. Rodriguez-Morales,
Infectious Diseases Series Editor

ISSN 2631-6188
ISBN 978-0-85466-394-1

Published in London, UK

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