

Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

## Chapter 10

# Emerging viral infections

Daisy D. Colón-López, Christopher P. Stefan, Jeffrey W. Koehler

*Diagnostic Systems Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, United States.*

### Introduction

Human populations living in close contact with their environment have continuously been exposed to emerging and reemerging infectious diseases. In many cases, localized outbreaks occur [1–4] that can lead to occasional, large scale epidemics and pandemics [5–8]. A well-known example of an emerging virus resulting in a pandemic is the human immunodeficiency virus (HIV-1). After jumping to humans from chimpanzees in Cameroon [9], this virus was able to maintain sustained, undetected person-to-person transmission in Kinshasa in the 1920s due to a variety of factors including high population densities [10]. The mobility of the population by well-connected railways allowed the virus to spread within Africa, and changing social behaviors such as injectable drug use and the sex trade allowed the virus to explode into a pandemic [10].

As mobile human populations become increasingly dense at this pathogen/host/environment interface, the risk of localized outbreaks becoming regional or world-wide epidemics is increasing. For instance, the 2014–2016 Ebola virus (Fig. 1) outbreak in West Africa originated in a rural region of Guinea [2]; travel, population density, and regional customs allowed the outbreak to become the largest known Ebola virus outbreak with multiple cases reaching other countries including Senegal [11], Nigeria [12], Mali [13], the United Kingdom [14], Italy [15], Spain [16], and the United States [17]. Of particular concern was a localized transmission chain in Lagos, Nigeria, that could have had an explosive impact in that country [12,18,19].

Similarly, Zika virus was first identified in 1947 [20] and caused limited infections [3,4] until its emergence in Micronesia in 2007 [5], French Polynesia in 2013 [6], and the Americas in 2015 [7,8]. In both of these cases, numerous factors had a role in enabling the rapid viral transmission of previously isolated infectious diseases. Besides increasing population density and ease of transportation, climate and ecological changes that expand a vector's range or habitat can lead to novel epidemics. For example, infections of Lassa virus, spread by

the *Mastomys natalensis* rat, are increased in West Africa during the dry seasons when the rats are more likely to be inside individuals' houses [21].

Fluctuations in climate resulting in increasing crop yields, and the associated rat population, followed by a severe drought can lead to increased cases of Lassa fever. Furthermore, climactic changes are altering vector-borne infectious disease risks due to expanded vector ranges. For example, there is an increased risk of Lyme disease in Canada due to tick range expansion [22], and climate change is expanding the mosquito range, and the associated infectious disease risk, of *Aedes albopictus* in the United States [23].

RNA viruses represent a significant source of viral outbreaks due partly to the higher mutation rate and error prone nature of RNA replication itself [24]. Newly introduced mutations into a viral genome can confer new pathogenic characteristics such as enabling transmission to new species or increased virulence. For instance, mutations within the severe acute respiratory syndrome coronavirus (SARS-CoV) genome allowed transmission from bats to humans [24,25]. The better understanding about the viral agents responsible of causing outbreaks in humans and the underlying factors driving emergence, the better the chances of preparedness and effective intervention for prevention of a new global epidemic (Figs. 1 and 2).

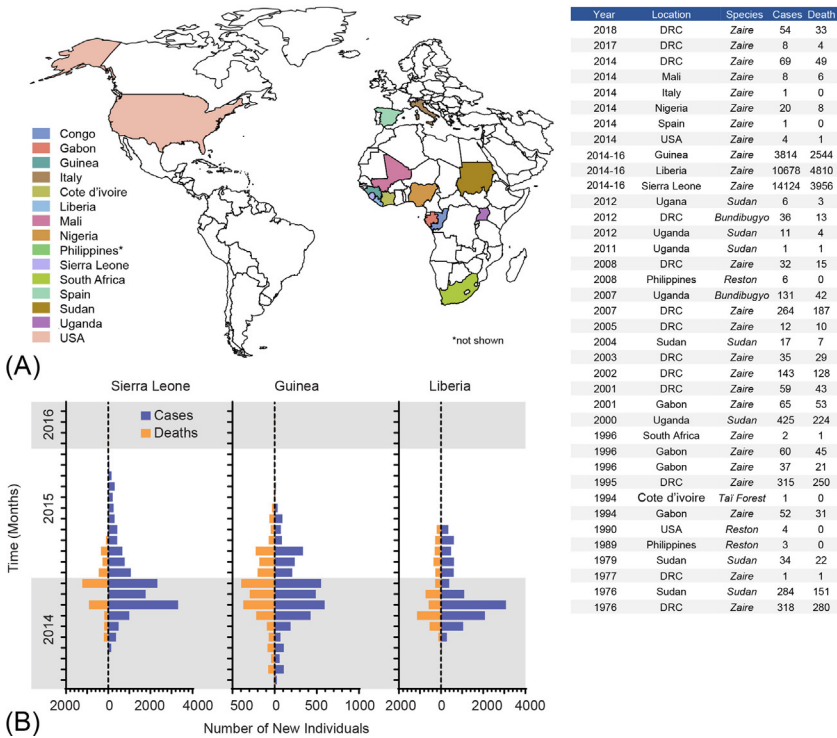
This chapter provides an overview of the genomics and precision medicine methodologies relevant to the topic of emerging and re-emerging viral threats. The chapter is divided up into two main sections: viral genomics and host genomics, and include examples of their applications in pathogen detection, population susceptibility, diagnostics, and outbreak response.

## Viral genomics

The seminal investigations on the transmission of yellow fever virus by Walter Reed and colleagues in 1901 [27,28] ushered in an era of discovery and classification of a wide variety of viral pathogens including smallpox virus, poliovirus, and measles virus. Technological advancements including viral cell culture, plaque assays, enzyme-linked immunosorbent assays, and monoclonal antibodies pushed human virology forward. A second wave of viral discovery and characterization is currently ongoing, harvesting the power of next-generation sequencing to identify unknown viruses in acute febrile illness patients [29–31]. Increasingly, efforts are underway to identify the next emerging pathogen by sequencing and identifying the viruses circulating at the human/animal/pathogen interface [32,33]. While detection of viruses in wildlife does not predict transmission, identifying mutations or gene acquisitions through surveillance and continued discovery will provide valuable insight in the future.

## Pathogen discovery

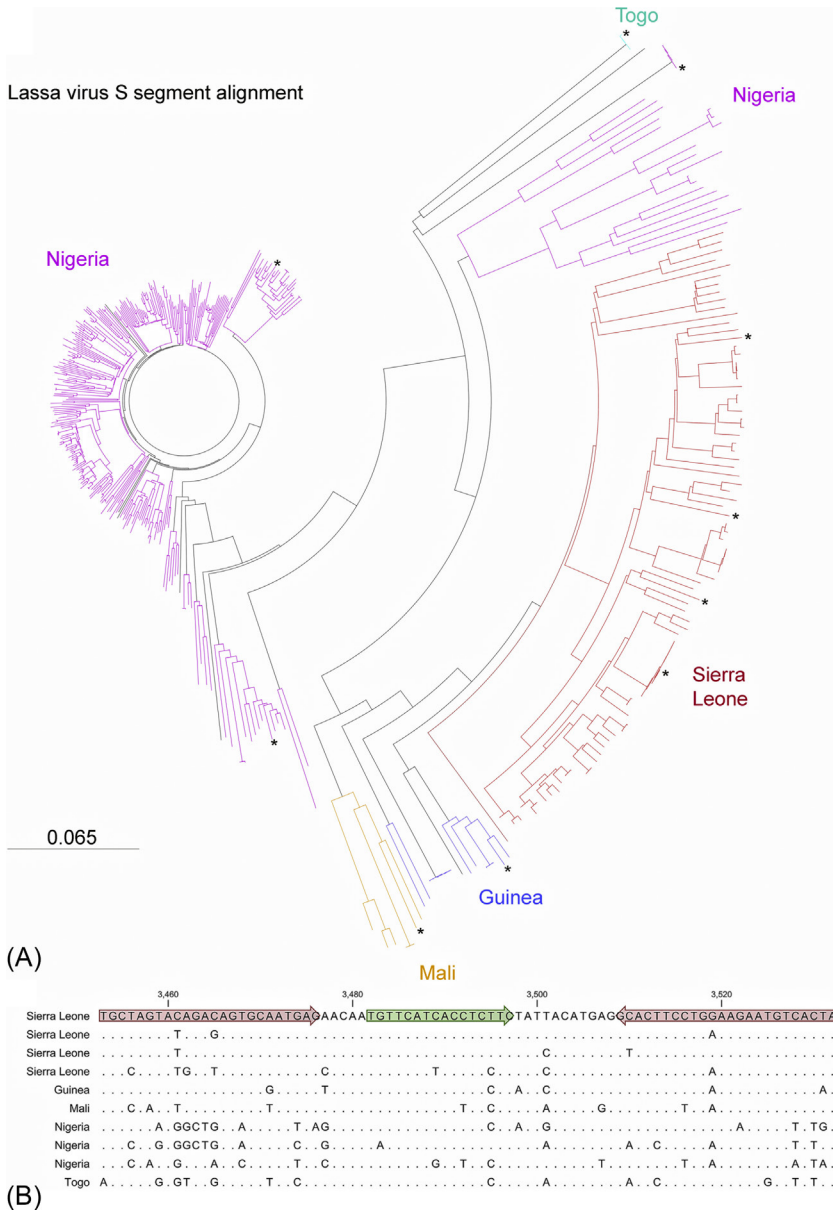
A major hindrance for precision medicine toward emerging zoonotic pathogens is the limited global characterization of potential threats. Taxonomic placement



**FIG. 1** Ebola virus outbreaks overtime. (A) Global map showing the countries where Ebola virus cases and deaths have been reported over the last 42 years. The information about each outbreak is shown on the right. As of November 2018, there is an ongoing Ebola virus outbreak in DRC and the data on the table does not reflect the final outcome. (B) The graphs show the number of new cases and deaths per month during the 2014 Ebola virus outbreaks in Sierra Leone, Guinea, and Liberia. Panel (A): Data adapted from [www.cdc.gov](http://www.cdc.gov). Panel (B): Data adapted from [www.who.int](http://www.who.int).

of viruses is problematic due to the lack of universal genes, such as the bacterial 16S RNA gene, limiting classification to viruses with defined biological characteristics [34]. In fact a mere 5000 total species are currently classified by the International Committee on Taxonomy of Viruses (ICTV) which pales in comparison to the estimated 320,000 viral species infecting mammals alone [35]. Metagenomic sequencing has highlighted this significant gap in classified vs unknown viromes, and while biological characterization will not also always be defined, knowledge can be gained from genomic sequences including evolutionary relationships and genome structure [34]. Continuing to gather viral genomic information from environmental, plant, and animal samples will continue to fill gaps in our knowledge base and further prepare us for detecting and predicting emerging pathogens.

Metagenomic sequencing has been used for the discovery of previously unclassified human pathogens. In 2008 Lujo virus was discovered in South Africa



**FIG. 2** Sequence diversity of Lassa virus complicates molecular diagnostic tool development. A phylogenetic tree (A) was generated using an alignment of full length and near full length Lassa virus S segment nucleic acid sequences available in GenBank. Diversity of Lassa virus is linked to geographic location as shown here for viruses sequenced from Nigeria, Sierra Leone, Guinea, Mali, and Togo. Selected sequences from each country [starred in (A)] are aligned in (B). The forward and reverse primers (red arrows) and the probe (green arrow) from a previously published Lassa virus Josiah real-time RT-PCR assay [26] are indicated. The primers and probe are exact matches to the Lassa virus Josiah sequence; however, contain mismatches within other Lassa virus isolates, including ones found in Sierra Leone.

[31]. Five patients with undiagnosed hemorrhagic fever were discovered after air transfer of a critically ill index case. The disease resulted in a case fatality rate of 80%. Unbiased pyrosequencing of RNA extracts from human samples identified approximately 50% of an arenavirus genome which was thereafter gap filled using primers designed off the sequenced genome. Phylogenetic analysis confirmed the identification of a novel Old World arenavirus [31]. Similarly, deep sequencing identified Bas-Congo virus, of a novel rhabdovirus, in three human cases in the Democratic Republic of Congo [30]. This virus was associated with high fever and rapid death and was found to have only 34% amino-acid identity with other rhabdoviruses. The discovery and characterization of these viruses has allowed novel diagnostics to be developed, including real-time PCR, leading to a better preparedness for future outbreaks.

Sequencing human samples from outbreaks has resulted in the discovery of several viruses; however, 70% of emerging human pathogens result from wildlife [36]. Analysis of samples from non-human hosts including arthropods, bats, rodents, and domestic animals offers potential insight into the transmission, evolution, and treatment of these pathogens [37]. Deep sequencing on more than 220 invertebrate species resulted in the discovery of 1445 phylogenetically distinct viromes filling in phylogenetic and evolutionary gaps [38]. Characterization of bacterial and viral relationships in mosquito arthropods demonstrated a symbiotic relationship between the bacterium and host, limiting dengue virus infection and potentially revealing new antiviral strategies [39,40]. The discovery of Middle-East Respiratory Syndrome-Coronavirus (MERS-CoV) in camels demonstrated these animals as a potential reservoir and host for transmission [41,42]. While detection of viruses in wildlife does not predict transmission, identifying mutations or gene acquisitions through surveillance and continued discovery will provide valuable insight in the future.

## **Epidemiology and outbreak response**

In addition to pathogen discovery, metagenomic sequencing is increasingly used to monitor viral genomic changes as an outbreak is occurring [43–46]. The Ebola virus outbreak in West Africa resulted in 26,648 cases and 11,017 documented deaths, and genomic sequencing was applied in near real-time to provide information to aid in containing the outbreak [44,45]. Sequencing results early in the outbreak provided valuable insight into origination and transmission routes demonstrating the outbreak started from a single introduction in Guinea in December 2013 and was sustained by human to human transmissions [2]. Sequencing provided molecular evidence that Ebola virus was transmissible by sexual intercourse leading to changes in CDC recommendations for survivors [45,47] and establishing programs to support national testing of semen and other body fluids in male survivors [48]. Rapid outbreak sequencing allowed for the identification of transmission chains in sporadic clusters following the outbreak [46], further adding insight to end the epidemic.

Sequencing data from Sierra Leone early in the outbreak also found increased Ebola virus diversity that could have an impact on sequence-based therapeutics, vaccines, and diagnostic assays being fielded at the time [44]. Having such rapid sequencing information during an outbreak would inform responders about the potential efficacy of diagnostics and sequence-based countermeasures, either reassuring decision makers or informing them of the need to modify strategies based on the findings.

## Diagnostics and therapeutic response

While sequencing has found a niche in the discovery and epidemiologic tracing of emerging infections, its roll for diagnostics is still in its infancy. Emerging pathogens often occur in austere environments or countries with limited infrastructure not conducive to sequencing technologies. Sequencing still has significant hurdles in decreasing the technical and mechanical requirements for routine clinical use. However, next-generation sequencing offers limitless potential due to the necessity for little to no prior knowledge in sample composition. Newer technologies such as nanopore sequencing are looking to minimize these hurdles by creating smaller foot-prints and near real-time sequence analysis with minimal sample preparation [49]; however, difficult paths to their acceptance beyond laboratory derived tests to full regulatory approval remain.

Knowledge of viral genomic sequences in designing rapid point of care molecular diagnostics such as PCR is invaluable. The last decade has seen a significant increase in the use of PCR as a primary diagnostic due to its speed, sensitivity, and cost. However, due to the lack of available genomic sequences and high genetic variation within certain viral species, finding a conserved target can be difficult. The advent of multiplex PCR has allowed more targets to be captured in a single assay, lowering costs and increasing throughput. For example, this technique has been used to subtype influenza A and B viruses [50]. Other diagnostic devices such as the BioFire FilmArray can run multiple PCRs at once on a single device allowing the user to select between different panels [51–55]. Whether singleplex or multiplex, PCR remains the most rapid and sensitive method for the detection of viral genomes directly from clinical matrices.

Coordinating with detection, viral genome sequences are increasingly being used to predict and guide antiviral therapy. During the Ebola virus outbreak, sequence analysis of the viral genome over time demonstrated changes which could make the pathogen resistant to therapeutics such as siRNAs, phosphorodiamidate morpholino oligomers (PMOs), and antibodies [56]. The discovery of the CRISPR/Cas9 system and its ability to destroy dsDNA has brought to light its potential in mutating essential sites or removing proviral DNA from infected cells during and HIV infection CRISPR [57]. Similarly studies involving herpesviruses, large dsDNA viruses, have demonstrated the ability to destroy latent herpesvirus from infected cells [58,59]. These methods require prior knowledge of the viral genome promising a future where antiviral therapies are targeted specifically toward an individual's own specific infection.

## Host genomics

Emerging and re-emerging viral diseases pose unique challenges to the use of omics and precision medicine tools. How does one predict when and where a pathogen will jump a species barrier or emerge into a new population that could rapidly spread into new geographic regions? To further complicate the scenario, a new human viral infection may not present with obvious signs of an infection. Such a virus may get introduced and transmitted across populations while remaining asymptomatic or unrecognized for long periods of times, like the hepatitis C virus (HCV) and HIV [10], for instance.

## Viral infections and host responses

Viruses rely on their small-sized genomes to encode enough information to hijack the host's cellular machinery for target-cell recognition and entry, genome replication, protein synthesis, viral particle assembly, and propagation. Evolutionarily, hosts evolved conserved mechanisms to generate an immune response to detect and limit an infection and to prevent re-infection in the future. The early innate immune response functions to slow the infection once the immune system recognizes there is an infection (extensively reviewed in [60]) and guides the subsequent adaptive immune response to the pathogen.

Successful control of these immunological processes depend on tight control of the host's immunological and inflammatory pathways by regulating gene transcription. These processes result in measurable changes in the relative expression levels of coding and non-coding RNA and protein, even when an infection is asymptomatic [61–65] or difficult to diagnose [66]. The development of low-cost and less-time consuming genomics had facilitated the study of pathway-specific transcript alterations during viral infections. Consequently, various transcriptomic-based assays have been developed to study, characterize, and identify signs of infection using transcript signatures. For example, Zaas and colleagues identified a unique immunological gene expression profile capable of differentiating viral from bacterial respiratory infections in humans [64,65], laying to foundations for appropriate antibiotic use without direct pathogen identification.

## Host-based diagnostics and genomics approaches

Traditionally, diagnosing viral infections is made based on clinical symptoms, PCR and serological testing, and virus isolation. While the world of omics is expanding with new and improved platforms, host transcriptome-based assays are becoming more feasible and widely available. Those of more relevance to this chapter are targeted and agnostic next-generation sequencing (NGS) platforms for genome-wide association studies (GWAS) and transcriptomics as well as microarray-based assays for amplification-independent profiling of transcripts. GWAS have direct applications to precision medicine whereas host-based



transcriptomic assays have shown to have multiple applications for clinical diagnosis, and pathogen identification and characterization. These technologies were not specifically developed for emerging or re-emerging viruses but can be leveraged for these purposes based on their applications.

GWAS may provide insights into the genetic factors driving population susceptibility to viral infections [67,68]. This information can be utilized to identify populations within geographic regions at higher or lower risk of being infected with a new pathogen. Higher-risk populations may include those with increased direct exposure to animal reservoirs of zoonotic pathogens or their arthropod vectors. Other population subgroups without defined topographical relationships can be determined as well. A few examples of these are demographic, age and, gender groups that can be classified based on genetic polymorphisms and other susceptibility markers. In this setting, GWAS can provide guidance in outbreak preparedness and intervention.

### Transcriptomic methods

The information provided by GWAS-based studies is not focused on the directed response to an infection but on the information already stored within the host's genome. On the other hand, transcriptomic approaches identify the interactions between the pathogen and the host's genome by evaluating transcript levels, typically mRNAs. The most commonly used transcriptomic methods are RNA-seq [69,70] and microarrays [65,66]. RNA-seq is generally suitable for unbiased transcriptomic profiling and provides better understanding of global transcriptomic changes. This agnostic method is appropriate for identifying changes in the human transcriptome as a result of an emerging viral infection to show specific mechanisms of immune response evasion and other effects in the host's biology at the transcriptomic level. Conversely, targeted approaches depend on previous knowledge of host-transcriptomics.

Studies with human cohorts and animal models have developed gene signatures that discriminate between viral and bacterial respiratory infections [61–65]. Other groups have developed sepsis classifiers to guide treatment options in clinical settings [71,72]. A goal of developing these disease classifiers is to use them to predict disease outcome prior to the onset of symptoms and to assist in decision making when a symptomatic disease with an unknown etiology is suspected. Effective and accurate discernment between a viral and a bacterial infection can make the difference in administering the appropriate therapeutics and potentially saving lives. When a specific viral species can't be identified, a gene classifier may be useful to determine potential disease outcomes such as lethality.

### Closing remarks

As technology continues to advance, the linkages of genomics and precision medicine with emerging infectious diseases will continue to strengthen. Improvements with sequencing accuracy and speed are pushing pathogen-specific

genomics to near real-time [73,74], allowing rapid access to critical information within a timeframe that can impact an outbreak response [44,45]. Confidence in the results from sequencing is leading to clinically actionable diagnostic patient testing [29], and it is reasonable to foresee rapid, pathogen agnostic diagnostic assays routinely utilized in the clinic.

Population growth, expansion into rural geographic regions, and rapid travel has greatly expanded the pathogen/host/environment interactome, putting a greater number of people at risk for formerly exotic infectious diseases such as Ebola virus or even the next unknown, soon-to-emerge pathogen. Applying genomics and the host transcriptome to generalize the response to a viral or bacterial pathogen would allow for more appropriate antibiotic use without requiring direct pathogen detection. Besides the benefits to antibiotic stewardship in an era where antimicrobial resistance is expanding rapidly, such an application can greatly improve clinical care in the event of an emerging, highly virulent unknown organism.

## Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

## References

- [1] Report of an International Commission. Ebola haemorrhagic fever in Zaire. Bull World Health Organ 1976;56(2):271–93. 1978, 307456. PubMed Central PMCID. PMC2395567.
- [2] Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, et al. Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med 2014;371(15):1418–25. <https://doi.org/10.1056/NEJMoa1404505>, 24738640.
- [3] Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Trans R Soc Trop Med Hyg 1954;48(2):139–45. 13157159.
- [4] Olson JG, Ksiazek TG, Suhandiman T. Zika virus, a cause of fever in Central Java, Indonesia. Trans R Soc Trop Med Hyg 1981;75(3):389–93, 6275577.
- [5] Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, federated states of Micronesia. N Engl J Med 2009;360(24):2536–43. <https://doi.org/10.1056/NEJMoa0805715>, 19516034.
- [6] Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French polynesia, South pacific, 2013. Emerg Infect Dis 2014;20(6):1085–6. <https://doi.org/10.3201/eid2006.140138>, 24856001. PubMed Central PMCID. PMC4036769.
- [7] Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. Emerg Infect Dis 2015;21(10):1885–6. <https://doi.org/10.3201/eid2110.150847>, 26401719. PubMed Central PMCID. PMC4593454.
- [8] Hennessey M, Fischer M, Staples JE. Zika virus spreads to new areas—region of the Americas, may 2015–january 2016. MMWR Morb Mortal Wkly Rep 2016;65(3):55–8. <https://doi.org/10.15585/mmwr.mm6503e1>, 26820163.
- [9] Keele BF, Van Heuverswyn F, Li Y, Bailes E, Takehisa J, Santiago ML, et al. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. Science 2006;313(5786):523–6. <https://doi.org/10.1126/science.1126531>, 16728595. PubMed Central PMCID. PMC4242710.

- [10] Faria NR, Rambaut A, Suchard MA, Baele G, Bedford T, Ward MJ, et al. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science* 2014;346(6205):56–61. <https://doi.org/10.1126/science.1256739>, [25278604](https://pubmed.ncbi.nlm.nih.gov/25278604/). PubMed Central PMCID. PMCPMC4254776.
- [11] Mirkovic K, Thwing J, Diack PA. Centers for disease C, prevention. Importation and containment of Ebola virus disease—Senegal, august–september 2014. *MMWR Morb Mortal Wkly Rep* 2014;63(39):873–4, [25275333](https://pubmed.ncbi.nlm.nih.gov/25275333/). PubMed Central PMCID. PMCPMC4584878.
- [12] Shuaib F, Gunnala R, Musa EO, Mahoney FJ, Oguntimehin O, Nguku PM, et al. Ebola virus disease outbreak—Nigeria, july–september 2014. *MMWR Morb Mortal Wkly Rep* 2014;63(39):867–72, [25275332](https://pubmed.ncbi.nlm.nih.gov/25275332/). PubMed Central PMCID. PMCPMC4584877.
- [13] Diarra B, Safronetz D, Sarro YD, Kone A, Sanogo M, Tounkara S, et al. Laboratory response to 2014 Ebola virus outbreak in Mali. *J Infect Dis* 2016;214(suppl 3):S164–8. <https://doi.org/10.1093/infdis/jiw200>, [27707892](https://pubmed.ncbi.nlm.nih.gov/27707892/). PubMed Central PMCID. PMCPMC5050465.
- [14] Gulland A. Second Ebola patient is treated in UK. *BMJ* 2014;349:g7861. <https://doi.org/10.1136/bmj.g7861>, [25552628](https://pubmed.ncbi.nlm.nih.gov/25552628/).
- [15] Castilletti C, Carletti F, Gruber CE, Bordi L, Lalle E, Quartu S, et al. Molecular characterization of the first Ebola virus isolated in Italy, from a health care worker repatriated from Sierra Leone. *Genome Announc* 2015;3(3):e00639-15. <https://doi.org/10.1128/genomeA.00639-15>, [26089420](https://pubmed.ncbi.nlm.nih.gov/26089420/). PubMed Central PMCID. PMCPMC4472897.
- [16] Lopaz MA, Amela C, Ordobas M, Dominguez-Berjon MF, Alvarez C, Martinez M, et al. First secondary case of Ebola outside Africa: epidemiological characteristics and contact monitoring, Spain, september to november 2014. *Euro Surveill* 2015;20(1):21003, [25613651](https://pubmed.ncbi.nlm.nih.gov/25613651/).
- [17] McCarthy M. Second US nurse with Ebola had traveled by plane. *BMJ* 2014;349:g6277. <https://doi.org/10.1136/bmj.g6277>, [25324208](https://pubmed.ncbi.nlm.nih.gov/25324208/).
- [18] Yusuf I, Adam RU, Ahmad SA, Yee PL. Ebola and compliance with infection prevention measures in Nigeria. *Lancet Infect Dis* 2014;14(11):1045–6. [https://doi.org/10.1016/S1473-3099\(14\)70954-5](https://doi.org/10.1016/S1473-3099(14)70954-5), [25282666](https://pubmed.ncbi.nlm.nih.gov/25282666/).
- [19] Fasina FO, Shittu A, Lazarus D, Tomori O, Simonsen L, Viboud C, et al. Transmission dynamics and control of Ebola virus disease outbreak in Nigeria, july to september 2014. *Euro Surveill* 2014;19(40):20920, [25323076](https://pubmed.ncbi.nlm.nih.gov/25323076/).
- [20] Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;46(5):509–20. [12995440](https://pubmed.ncbi.nlm.nih.gov/12995440/).
- [21] Fichet-Calvet E, Lecompte E, Koivogui L, Soropogui B, Dore A, Kourouma F, et al. Fluctuation of abundance and Lassa virus prevalence in *Mastomys natalensis* in Guinea, West Africa. *Vector Borne Zoonotic Dis* 2007;7(2):119–28. <https://doi.org/10.1089/vbz.2006.0520>, [17627428](https://pubmed.ncbi.nlm.nih.gov/17627428/).
- [22] Kulkarni MA, Berrang-Ford L, Buck PA, Drebot MA, Lindsay LR, Ogden NH. Major emerging vector-borne zoonotic diseases of public health importance in Canada. *Emerg Microbes Infect* 2015;4:e33. <https://doi.org/10.1038/emi.2015.33>, [26954882](https://pubmed.ncbi.nlm.nih.gov/26954882/). PubMed Central PMCID. PMCPMC4773043.
- [23] Rochlin I, Ninivaggi DV, Hutchinson ML, Farajollahi A. Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: implications for public health practitioners. *PLoS One* 2013;8(4):e60874. <https://doi.org/10.1371/journal.pone.0060874>, [23565282](https://pubmed.ncbi.nlm.nih.gov/23565282/). PubMed Central PMCID. PMCPMC3614918.
- [24] Howard CR, Fletcher NF. Emerging virus diseases: can we ever expect the unexpected? *Emerg Microbes Infect* 2012;1(12):e46. <https://doi.org/10.1038/emi.2012.47>, [26038413](https://pubmed.ncbi.nlm.nih.gov/26038413/). PubMed Central PMCID. PMCPMC3630908.
- [25] Bolles M, Donaldson E, Baric R. SARS-CoV and emergent coronaviruses: viral determinants of interspecies transmission. *Curr Opin Virol* 2011;1(6):624–34. <https://doi.org/10.1016/j.coviro.2011.10.012>, [22180768](https://pubmed.ncbi.nlm.nih.gov/22180768/). PubMed Central PMCID. PMCPMC3237677.

- [26] Trombley AR, Wachter L, Garrison J, Buckley-Beason VA, Jahrling J, Hensley LE, et al. Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and new world hantaviruses. *Am J Trop Med Hyg* 2010;82(5):954–60. <https://doi.org/10.4269/ajtmh.2010.09-0636>. 20439981. PubMed Central PMCID. PMC2861391s.
- [27] Staples JE, Monath TP. Yellow fever: 100 years of discovery. *JAMA* 2008;300(8):960–2. <https://doi.org/10.1001/jama.300.8.960>. 18728272.
- [28] Reed W, Carroll J, Agramonte A. The etiology of yellow fever: an additional note. *JAMA* 1901;36:431–40.
- [29] Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med* 2014;370(25):2408–17. <https://doi.org/10.1056/NEJMoa1401268>. 24896819. PubMed Central PMCID. PMC4134948.
- [30] Grard G, Fair JN, Lee D, Slikas E, Steffen I, Muyembe JJ, et al. A novel rhabdovirus associated with acute hemorrhagic fever in central Africa. *PLoS Pathog* 2012;8(9):e1002924. <https://doi.org/10.1371/journal.ppat.1002924>. 23028323. PubMed Central PMCID. PMC3460624.
- [31] Briese T, Paweska JT, McMullan LK, Hutchison SK, Street C, Palacios G, et al. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from southern Africa. *PLoS Pathog* 2009;5(5):e1000455. <https://doi.org/10.1371/journal.ppat.1000455>. 19478873. PubMed Central PMCID. PMCPCMC2680969.
- [32] Goldstein T, Anthony SJ, Gbakima A, Bird BH, Bangura J, Tremeau-Bravard A, et al. Author correction: the discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nat Microbiol* 2018;3(10):1084–9. <https://doi.org/10.1038/s41564-018-0315-3>. PubMed PMID. 30410089.
- [33] Geldenhuys M, Mortlock M, Weyer J, Bezuidt O, Seamark ECJ, Kearney T, et al. A metagenomic viral discovery approach identifies potential zoonotic and novel mammalian viruses in *Neoromicia* bats within South Africa. *PLoS One* 2018;13(3):e0194527. <https://doi.org/10.1371/journal.pone.0194527>. 29579103. PubMed Central PMCID. PMCPCMC5868816.
- [34] Simmonds P, Adams MJ, Benko M, Breitbart M, Brister JR, Carstens EB, et al. Consensus statement: virus taxonomy in the age of metagenomics. *Nat Rev Microbiol* 2017;15(3):161–8. <https://doi.org/10.1038/nrmicro.2016.177>. 28134265.
- [35] Anthony SJ, Epstein JH, Murray KA, Navarrete-Macias I, Zambrana-Torrel CM, Solovyov A, et al. A strategy to estimate unknown viral diversity in mammals. *MBio* 2013;4(5):e00598–13. <https://doi.org/10.1128/mBio.00598-13>. 24003179. PubMed Central PMCID. PMCPCMC3760253.
- [36] Cutler SJ, Fooks AR, van der Poel WH. Public health threat of new, reemerging, and neglected zoonoses in the industrialized world. *Emerg Infect Dis* 2010;16(1):1–7. <https://doi.org/10.3201/eid1601.081467>. 20031035. PubMed Central PMCID. PMCPCMC2874344.
- [37] Temmam S, Davoust B, Berenger JM, Raoult D, Desnues C. Viral metagenomics on animals as a tool for the detection of zoonoses prior to human infection? *Int J Mol Sci* 2014;15(6):10377–97. <https://doi.org/10.3390/ijms150610377>. 24918293. PubMed Central PMCID. PMCPCMC4100157.
- [38] Shi M, Lin XD, Tian JH, Chen LJ, Chen X, Li CX, et al. Redefining the invertebrate RNA virosphere. *Nature* 2016;540(7634):539–43. <https://doi.org/10.1038/nature20167>. 27880757.
- [39] Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 2009;139(7):1268–78. <https://doi.org/10.1016/j.cell.2009.11.042>. 20064373.

- [40] Bian G, Xu Y, Lu P, Xie Y, Xi Z. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog* 2010;6(4):e1000833. <https://doi.org/10.1371/journal.ppat.1000833>. 20368968. PubMed Central PMCID. PMCPMC2848556.
- [41] Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infect Dis* 2014;14(2):140–5. [https://doi.org/10.1016/S1473-3099\(13\)70690-X](https://doi.org/10.1016/S1473-3099(13)70690-X). 24355866.
- [42] Briese T, Mishra N, Jain K, Zalmout IS, Jabado OJ, Karesh WB, et al. Middle East respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. *MBio* 2014;5(3):e01146-14. <https://doi.org/10.1128/mBio.01146-14>. 24781747. PubMed Central PMCID. PMCPMC4010836.
- [43] Rohde H, Qin J, Cui Y, Li D, Loman NJ, Hentschke M, et al. Open-source genomic analysis of Shiga-toxin-producing *E. coli* O104:H4. *N Engl J Med* 2011;365(8):718–24. <https://doi.org/10.1056/NEJMoa1107643>. 21793736.
- [44] Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. *Science* 2014;345(6202):1369–72. <https://doi.org/10.1126/science.1259657>. 25214632. PubMed Central PMCID. PMC4431643.
- [45] Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, et al. Molecular evidence of sexual transmission of Ebola virus. *N Engl J Med* 2015;373(25):2448–54. <https://doi.org/10.1056/NEJMoa1509773>. 26465384. PubMed Central PMCID. PMCPMC4711355.
- [46] Arias A, Watson SJ, Asogun D, Tobin EA, Lu J, Phan MVT, et al. Rapid outbreak sequencing of Ebola virus in Sierra Leone identifies transmission chains linked to sporadic cases. *Virus Evol* 2016;2(1):vew016. <https://doi.org/10.1093/ve/vew016>. 28694998. PubMed Central PMCID. PMCPMC5499387.
- [47] Christie A, Davies-Wayne GJ, Cordier-Lassalle T, Blackley DJ, Laney AS, Williams DE, et al. Possible sexual transmission of Ebola virus—Liberia, 2015. *MMWR Morb Mortal Wkly Rep* 2015;64(17):479–81. 25950255.
- [48] Purpura LJ, Soka M, Baller A, White S, Rogers E, Choi MJ, et al. Implementation of a National semen testing and counseling program for male Ebola survivors—Liberia, 2015–2016. *MMWR Morb Mortal Wkly Rep* 2016;65(36):963–6. <https://doi.org/10.15585/mmwr.mm6536a5>. 27632552.
- [49] Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. *Genomics* 2016;107(1):1–8. <https://doi.org/10.1016/j.ygeno.2015.11.003>. 26554401. PubMed Central PMCID. PMCPMC4727787.
- [50] Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. Multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. *J Clin Microbiol* 1998;36(10):2990–5. 9738055. PubMed Central PMCID. PMCPMC105099.
- [51] Ruggiero P, McMillen T, Tang YW, Babady NE. Evaluation of the BioFire FilmArray respiratory panel and the GenMark eSensor respiratory viral panel on lower respiratory tract specimens. *J Clin Microbiol* 2014;52(1):288–90. <https://doi.org/10.1128/JCM.02787-13>. 24131685. PubMed Central PMCID. PMCPMC3911424.
- [52] Gyawali P, Croucher D, Hewitt J. Preliminary evaluation of BioFire FilmArray((R)) gastrointestinal panel for the detection of noroviruses and other enteric viruses from wastewater and shellfish. *Environ Sci Pollut Res Int* 2018;25(27):27657–61. <https://doi.org/10.1007/s11356-018-2869-2>. 30083906.
- [53] Pulido MR, Moreno-Martinez P, Gonzalez-Galan V, Fernandez Cuenca F, Pascual A, Garnacho-Montero J, et al. Application of BioFire FilmArray blood culture identification

- panel for rapid identification of the causative agents of ventilator-associated pneumonia. *Clin Microbiol Infect* 2018;24(11):1213 e1–4. <https://doi.org/10.1016/j.cmi.2018.06.001.29906599>.
- [54] Soucek DK, Dumkow LE, VanLangen KM, Jameson AP. Cost justification of the BioFire FilmArray meningitis/encephalitis panel versus standard of care for diagnosing meningitis in a community hospital. *J Pharm Pract* 2019;32(1):36–40. <https://doi.org/10.1177/0897190017737697.897190017737697.29092659>.
- [55] Gay-Andrieu F, Magassouba N, Picot V, Phillips CL, Peyrefitte CN, Dacosta B, et al. Clinical evaluation of the BioFire FilmArray((R)) BioThreat-E test for the diagnosis of Ebola virus disease in Guinea. *J Clin Virol* 2017;92:20–4. <https://doi.org/10.1016/j.jcv.2017.04.015.28505570>.
- [56] Kugelman JR, Sanchez-Lockhart M, Andersen KG, Gire S, Park DJ, Sealfon R, et al. Evaluation of the potential impact of Ebola virus genomic drift on the efficacy of sequence-based candidate therapeutics. *MBio* 2015;6(1):e02227-14. <https://doi.org/10.1128/mBio.02227-14.25604787>. PubMed Central PMCID. PMC4313914.
- [57] Soppe JA, Lebbink RJ. Antiviral goes viral: harnessing CRISPR/Cas9 to combat viruses in humans. *Trends Microbiol* 2017;25(10):833–50. <https://doi.org/10.1016/j.tim.2017.04.005.28522157>.
- [58] Wang J, Quake SR. RNA-guided endonuclease provides a therapeutic strategy to cure latent herpesviridae infection. *Proc Natl Acad Sci U S A* 2014;111(36):13157–62. <https://doi.org/10.1073/pnas.1410785111.25157128>. PubMed Central PMCID. PMC4246930.
- [59] van Diemen FR, Kruse EM, Hooykaas MJ, Bruggeling CE, Schurch AC, van Ham PM, et al. CRISPR/Cas9-mediated genome editing of herpesviruses limits productive and latent infections. *PLoS Pathog* 2016;12(6):e1005701. <https://doi.org/10.1371/journal.ppat.1005701.27362483>. PubMed Central PMCID. PMC4928872.
- [60] Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev* 2001;14(4):778–809. table of contents. Epub 2001/10/05, <https://doi.org/10.1128/CMR.14.4.778-809.200111585785>.
- [61] McClain MT, Henaou R, Williams J, Nicholson B, Veldman T, Hudson L, et al. Differential evolution of peripheral cytokine levels in symptomatic and asymptomatic responses to experimental influenza virus challenge. *Clin Exp Immunol* 2016;183(3):441–51. <https://doi.org/10.1111/cei.12736.26506932>. PubMed Central PMCID. PMC4750592.
- [62] McClain MT, Nicholson BP, Park LP, Liu TY, Hero 3rd AO, Tsalik EL, et al. A genomic signature of influenza infection shows potential for presymptomatic detection, guiding early therapy, and monitoring clinical responses. *Open Forum Infect Dis* 2016;3(1):ofw007. <https://doi.org/10.1093/ofid/ofw007.26933666>. PubMed Central PMCID. PMC4771939.
- [63] Woods CW, McClain MT, Chen M, Zaas AK, Nicholson BP, Varkey J, et al. A host transcriptional signature for presymptomatic detection of infection in humans exposed to influenza H1N1 or H3N2. *PLoS One* 2013;8(1):e52198. <https://doi.org/10.1371/journal.pone.0052198.23326326>. PubMed Central PMCID. PMC3541408.
- [64] Zaas AK, Burke T, Chen M, McClain M, Nicholson B, Veldman T, et al. A host-based RT-PCR gene expression signature to identify acute respiratory viral infection. *Sci Transl Med* 2013;5(203):203ra126. <https://doi.org/10.1126/scitranslmed.3006280.24048524>. PubMed Central PMCID. PMC4286889.
- [65] Zaas AK, Chen M, Varkey J, Veldman T, Hero 3rd AO, Lucas J, et al. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans. *Cell Host Microbe* 2009;6(3):207–17. <https://doi.org/10.1016/j.chom.2009.07.006.19664979>. PubMed Central PMCID. PMC2852511.

- [66] Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, et al. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study. *PLoS Med* 2013;10(10):e1001538. <https://doi.org/10.1371/journal.pmed.1001538>. 24167453. PubMed Central PMCID. PMC3805485.
- [67] Zheng R, Li Z, He F, Liu H, Chen J, Chen J, et al. Genome-wide association study identifies two risk loci for tuberculosis in Han Chinese. *Nat Commun* 2018;9(1):4072. <https://doi.org/10.1038/s41467-018-06539-w>. 30287856. PubMed Central PMCID. PMC6172286.
- [68] Compaore TR, Soubeiga ST, Ouattara AK, Obiri-Yeboah D, Tchelougou D, Maiga M, et al. APOBEC3G variants and protection against HIV-1 Infection in Burkina Faso. *PLoS One* 2016;11(1):e0146386. <https://doi.org/10.1371/journal.pone.0146386>. 26741797. PubMed Central PMCID. PMC4704832.
- [69] Speranza E, Bixler SL, Altamura LA, Arnold CE, Pratt WD, Taylor-Howell C, et al. A conserved transcriptional response to intranasal Ebola virus exposure in nonhuman primates prior to onset of fever. *Sci Transl Med* 2018;10(434):eaq1016. <https://doi.org/10.1126/scitranslmed.aag1016>. 29593102.
- [70] Speranza E, Altamura LA, Kulcsar K, Bixler SL, Rossi CA, Schoepp RJ, et al. Comparison of transcriptomic platforms for analysis of whole blood from Ebola-infected *Cynomolgus* Macaques. *Sci Rep* 2017;7(1):14756. <https://doi.org/10.1038/s41598-017-15145-7>. 29116224. PubMed Central PMCID. PMC5676990.
- [71] Sweeney TE, Perumal TM, Henao R, Nichols M, Howrylak JA, Choi AM, et al. A community approach to mortality prediction in sepsis via gene expression analysis. *Nat Commun* 2018;9(1):694. <https://doi.org/10.1038/s41467-018-03078-2>. PubMed PMID 29449546. PubMed Central PMCID. PMC5814463.
- [72] Sweeney TE, Wong HR, Khatri P. Robust classification of bacterial and viral infections via integrated host gene expression diagnostics. *Sci Transl Med* 2016;8(346):346ra91. <https://doi.org/10.1126/scitranslmed.aaf7165>. 27384347. PubMed Central PMCID. PMC5348917.
- [73] Hoenen T, Groseth A, Rosenke K, Fischer RJ, Hoenen A, Judson SD, et al. Nanopore sequencing as a rapidly deployable Ebola outbreak tool. *Emerg Infect Dis* 2016;22(2):331–4. <https://doi.org/10.3201/eid2202.151796>. 26812583. PubMed Central PMCID. PMC4734547.
- [74] Walter MC, Zwirgmaier K, Vette P, Holowachuk SA, Stoecker K, Genzel GH, et al. MinION as part of a biomedical rapidly deployable laboratory. *J Biotechnol* 2017;250:16–22. <https://doi.org/10.1016/j.jbiotec.2016.12.006>. 27939320.