Are RNA Viruses Candidate Agents for the Next Global Pandemic? A Review

R. Carrasco-Hernandez, Rodrigo Jácome, Yolanda López Vidal, and Samuel Ponce de León

R. Carrasco-Hernandez, PhD, is a postdoctoral research fellow at the Microbiome Laboratory in the Postgraduate Division of the Faculty of Medicine at the Universidad Nacional Autónoma de México, CDMX., Rodrigo Jácome, MD, PhD, is a postdoctoral research fellow at the Microbiome Laboratory in the Postgraduate Division of the Faculty of Medicine at the Universidad Nacional Autónoma de México, CDMX., Yolanda López-Vidal, MD, PhD, is an associate professor "C" and is responsible for the Program of Microbial Molecular Immunology in the Department of Microbiology and Parasitology of the Faculty of Medicine at the Universidad Nacional Autónoma de México, CDMX., and Samuel Ponce-de-León, MD, MSc, is an associate professor "C", is responsible for the Microbiome Laboratory and Coordinator of the University Program for Health Research of the Faculty of Medicine at the Universidad Nacional Autónoma de México, CDMX

Address correspondence and reprint requests to Dr. R Carrasco-Hernandez, Laboratorio de Microbioma, Edificio "H" 4to piso, Facultad de Medicina, Circuito Interior, Ciudad Universitaria, Av. Universidad 3000, CP 04510, Ciudad de México, México or email: rcarrash@yahoo.com.mx.

Abstract

Pathogenic RNA viruses are potentially the most important group involved in zoonotic disease transmission, and they represent a challenge for global disease control. Their biological diversity and rapid adaptive rates have proved to be difficult to overcome and to anticipate by modern medical technology. Also, the anthropogenic change of natural ecosystems and the continuous population growth are driving increased rates of interspecies contacts and the interchange of pathogens that can develop into global pandemics. The combination of molecular, epidemiological, and ecological knowledge of RNA viruses is therefore essential towards the proper control of these emergent pathogens. This review outlines, throughout different levels of complexity, the problems posed by RNA viral diseases, covering some of the molecular mechanisms allowing them to adapt to new host species—and to novel pharmaceutical developments—up to the known ecological processes involved in zoonotic transmission.

Key words: Ebola; emerging infectious diseases; global pandemics; RNA viruses; SARS; zoonoses

"Variability is not actually caused by man; he only unintentionally exposes organic beings to new conditions of life, and then nature acts on the organization and causes it to vary."

—Charles Darwin

Introduction

The continuous growth of the human population closely linked to globalization, trade, and habitat fragmentation increasingly promote contact between people, domestic animals, and wildlife populations. Such contact between formerly isolated populations increases the risk of transmission of parasites to which they had not been exposed before. The increasing human interaction with wild environments has induced a number of pandemics originated from wildlife reservoirs, as was seen with the emergence of the Human Immunodeficiency Virus (HIV), H1N1 influenza, the highly pathogenic H5N1 avian influenza Nipah, Hendra, the Severe Acute Respiratory Syndrome Coronavirus

(SARS), and the recent Ebola virus (EBOV) (Feldmann 2014; Jones et al. 2008; Joseph et al. 2016).

Among all potential pathogens that may be involved in interspecies transmissions, RNA viruses are of special concern. In particular, RNA viruses have become important zoonotic agents originating from wildlife. Studies from the last decades have placed RNA viruses as primary etiological agents of human emerging pathogens, occupying up to 44% of all emerging infectious diseases (ranging from 25% to 44% in different studies), which, along with bacteria (10%–49%), overshadow other parasite groups such as fungi (7%–9%), protozoans (11%–25%), and helminths (3%–6%) (Binder et al. 1999; Jones et al. 2008; Morens et al. 2004; Woolhouse and Gowtage-Sequeria 2005). RNA viruses have higher probabilities to infect new host species because of their exceptionally shorter generation times and their faster evolutionary rates. The rapid evolutionary rates of RNA viruses build from frequent error-prone replication cycles (Holmes 2009). Mutation rates of RNA viruses can occur—roughly—at rates of six orders of magnitude greater than those of their cellular hosts (Holmes 2009). Moreover, their mutability can even surpass that of some DNA viruses by up to five orders of magnitude (e.g., 1.5×10^{-3} mutations per nucleotide, per genomic replication [m/n/gr] in the single-stranded RNA phage Qβ10, versus 1.8×10^{-8} m/n/gr in the double-stranded DNA virus herpes simplex virus type 1; Duffy et al. 2008).

RNA viruses are often highlighted as the most common class of pathogens behind new human diseases, with a rate of 2 to 3 novel viruses being discovered each year (Rosenberg 2015). Moreover, Rosenberg suggests that this is a small number of novel viruses discovered each year, and that it is an artifact of inadequate surveillance in tropical and subtropical countries, where even established endemic pathogens are often misdiagnosed. The literature describes the recent emergence of interspecies-transmitted RNA viruses, such as Chikungunya (CHIKV) and Zika (ZIKV) viruses, which represent new global pandemics. CHIKV was first well documented in Asia in the 1950s, and it has infected millions (Tsetsarkin et al. 2016) and recently spread to the Americas in 2013 (Weaver and Forrester 2015). It was followed by ZIKV in 2015. Other viruses, in turn, have been known to cause epidemics since early human history, as is the case of avian influenza viruses (Taubenberger and Morens 2010), but they still continue to produce new strains of current concern for human health. Weber et al. (2016) wrote a useful systematic review on five zoonotic, highly communicable RNA viruses including Lassa fever, Ebolavirus, Middle East respiratory syndrome (MERS), SARS, and Influenza A virus (IAV) and described key information on their biology and epidemiology as well as provided control guidance for clinical settings. This type of information is essential towards the proper control of RNA-viral emergent diseases.

Herein are described some of the problems that RNA-viral diseases represent for current control efforts, with special attention to zoonotic RNA viruses. The first section will cover molecular mechanisms explaining the rapid adaptation of RNA viruses to new selective pressures. Section 2 covers some pharmaceutical developments and field survey strategies against those known and yet-to-be-known RNA-viral diseases. Next, Section 3 shows a few examples illustrating the natural history of relevant RNA viral epidemics. Finally, Section 4 discusses some ecological and anthropogenic factors that govern the development of these epidemics.

## The Molecular Mechanisms That Generate Variability in RNA Viruses

RNA viruses show remarkable capabilities to adapt to new environments and confront the different selective pressures they encounter. Selective pressures on viruses not only include their host's immune system and defense mechanisms but also the current artificial challenges devised by the biomedical community (i.e., antiviral drugs aimed at key viral proteins like HIV-1 reverse-transcriptase inhibitors and Hepatitis B and C virus protease inhibitors). Their peculiar rate of adaptive evolution arises from their exceptionally high mutation rates. Table 1 shows examples of the mutation rates of well-studied RNA viruses including dengue virus (DENV), influenza virus H3N2, and HIV-1 compared to other species of microorganisms, exemplifying the mutation rates of bacteria, fungi, and protozoans. For viruses and bacteria, these rates are usually calculated in vitro from counting the changing proportions of individual cells (or viral particles) expressing a certain phenotype such as drug resistance (Foster 2006; Lee et al. 2012; Schrag et al. 1999). This list in Table 1 is not extensive, because the units of mutation rates are not always comparable; these examples are those from the few studies that coincided for mutations-per-site-per-generation. Still, RNA viruses show much faster mutation rates than the other groups. It must also be acknowledged that mutation rates vary within any taxonomic group; for example, Sanjuán and Domingo-Calap (2016) reported rates from 27 viruses ranging from 10^{-8} to 10^{-6} nucleotide mutations per nucleotide per infected cell (m/n/c) for DNA viruses and from 10^{-6} to 10^{-3} m/n/c for RNA viruses.

Recollections of viral evolutionary rates (i.e., mutation and substitution rates) have shown that viruses move in a very wide range of mutability, with single-stranded RNA viruses in one end and double-stranded DNA viruses on the other (Duffy

Table 1 Examples of spontaneous mutation rates for microorganisms

Group	Organism	Mutation rate (mutations per site per generation)	Reference
RNA viruses	DENV	2.64×10^{-5}	Bennett et al. (2003)
	Influenza H3N2	1.35×10^{-5}	Herlocher et al. (2001)
	HIV-1	4×10^{-5}	Mansky (1996)
Bacteria	*Yersinia pestis*	1.7×10^{-10}	Vogler et al. (2013)
	Escherichia coli	2×10^{-10}	Lee et al. (2012)
	Free living bacteria	2×10^{-10} to 2×10^{-9}	Price and Arkin (2015)
Fungi	*Saccharomyces cerevisiae*	3.3×10^{-10}	Kondrashov and Kondrashov (2010)
Protozoa	*Plasmodiun falciparium*	1×10^{-9}	Bopp et al. (2013)
	Caenorhabditis elegans	$2·1 \times 10^{-8}$	Gilleard (2013)

S. cerevisiae has been reported as an emergent infectious disease (Muñoz et al. 2005; Pérez-Torrado and Querol 2015).
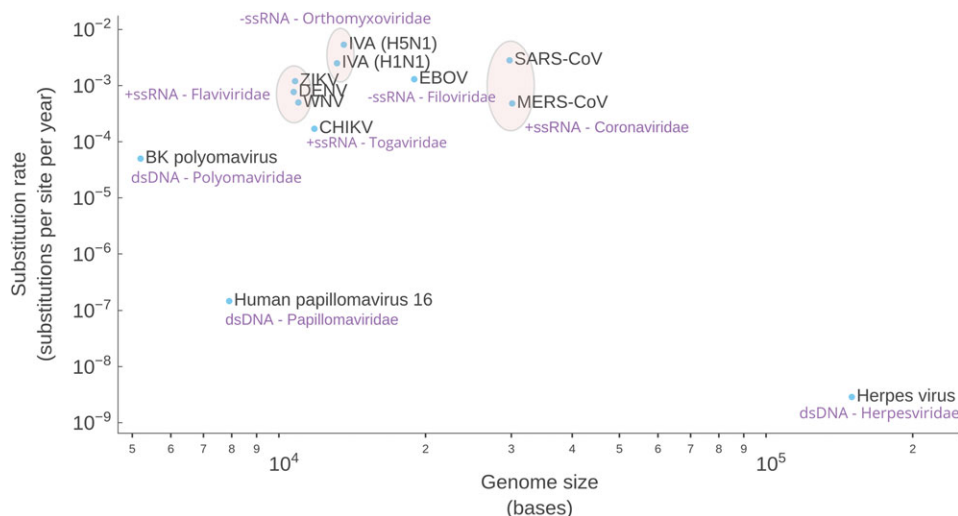
Figure 1 Genome size against substitution rate of DNA and RNA viruses. Genome size is given as the number of bases for single-stranded genomes, and as base pairs for double-stranded. Viruses and their corresponding abbreviations and references are as follows: RNA viruses: Dengue 2 virus (DENV; Afreen et al. 2016); Zika virus (ZIKV; Fajardo et al. 2016); Ebola virus (EBOV; Hoenen et al. 2015); Chikungunya virus (CHIKV; Cherian et al. 2009); Influenza virus A (H1N1) (IVA (H1N1); Klein et al. 2014); Influenza virus A (H5N1) (IVA (H5N1); Cattoli et al. 2011); SARS coronavirus (SARS-CoV; Lau et al. 2010); MERS-coronavirus (MERS-CoV; Zhang et al. 2016); West Nile virus (WNV; Añez et al. 2013). DNA viruses: Herpes virus McGeoch and Gatherer (2005); Human papillomavirus 16 (Zehender et al. 2016); BK polyomavirus (Chen et al. 2004). Corresponding type of genome is indicated: + or − = positive or negative sense; ss = single stranded; ds = double stranded. Corresponding family is also indicated, shaded areas are for viruses within the same family.

et al. 2008; Sanjuán and Domingo-Calap 2016). The substitution rates of many of the emergent RNA viruses have been calculated and follow the trend shown in Figure 1. Note that RNA viruses show greater substitution rates than DNA viruses. In spite of the fact that the mutation and substitution rates reflect the evolutionary rate of a biological entity, they are not equivalent (Duffy et al. 2008). The mutation rate is the raw measurement of the number of genetic changes such as point mutations and insertions/deletions that accumulate in time. The substitution rate can be defined as the number of mutations that are fixed per nucleotide site in time; therefore, it is a multifactorial measurement that considers the mutation rate, population size, generation time, and fitness. For a more profound analysis on the fundamental differences between these two evolutionary rates, their calculations, and an extensive comparison between DNA and RNA viruses, please refer to Duffy et al. 2008.

The Lack of Proofreading Ability of RNA Polymerases and Reverse Transcriptases

The single protein present in all RNA viruses and which seems to be homologous in all cases, at least from the crystal structures that have been obtained as of today (Jácome et al. 2015), is the RNA-dependent polymerase (either an RNA-dependent RNA polymerase or an RNA-dependent DNA polymerase, i.e., a reverse transcriptase). The majority of RNA viral replicases lack proofreading activity. In the case of replicative DNA polymerases of cellular organisms, an exonuclease is one of several proteins that correct the possible nucleotide misincorporations occurring during genome replication. In the absence of an exonuclease domain, the fidelity of the polymerases is determined by the steric constraints of the residues that form the active site. Ultimately, the absence of exonuclease activity increases the point mutation rate of the genome by omitting error correction during the replication process of RNA viruses.

When a correct nucleotide is bound to the active site, many favorable interactions occur. However, the incorporation of an incorrect nucleotide makes replication a less efficient process, the number of polymerase-nucleotide interactions diminishes, and the conformational changes are slower, deforming the catalytic site of the enzyme making the phosphoryl transfer reaction less proficient (Castro et al. 2005; Kunkel 2004). The error rates of viral RNA replicases are similar, albeit a little higher, to those of cellular DNA polymerases when the latter are devoid of their associated proofreading mechanisms. These similarities in error rates point towards inherent limits in terms of efficiency and fidelity in both DNA and RNA polymerases. However, the prerogatives for survival seem to be different. In the case of DNA-based cellular organisms with huge genomes in terms of length and gene content, the presence of multiple proofreading pathways diminishes the burden of random mutations, hence, preserving the genetic identity of the species. Nevertheless, RNA viruses are found at the opposite end, benefiting from mutability. As a matter of fact, RNA viral populations are considered to form quasispecies, that is, a swarm of genetic mutants revolving around a consensus sequence, in a phenomenon also known as "the survival of the flattest," implying that viruses with larger numbers of variants of a given sequence (i.e., a "flatter" curve of abundances) will have more probabilities to continue replicating inside the host (Holmes 2010; Lauring and Andino 2010). The RNA viral error rate is at the limit of mutation tolerability, and small increases in this rate generate what is known as mutational meltdown or error catastrophe, in which the viral fitness plummets down, leading to viral extinction (Lauring and Andino 2010; Novella et al. 2014). This principle is at the base of using mutagenic agents such as Ribavirin as antiviral drugs.

Interestingly, diminishing the viral mutation rate of a viral population may also result in detrimental loss of fitness. Different experimental conditions and point mutations have proven to alter polymerase fidelity. Higher temperatures (Álvarez and Menéndez-Arias 2014) and lower pH (Eckert and

Kunkel 1993) increase fidelity in the HIV reverse transcriptase (RT). Different studies, mostly on the Picornaviridae family polymerase, have shown the effects of point mutations in polymerase fidelity. An example of these effects is the G64S mutation in the poliovirus polymerase (Pfeiffer and Kirkegaard 2003). This poliovirus mutant proved to be a high-fidelity variant with less pathogenicity and an attenuated phenotype compared to the wild type (Pfeiffer and Kirkegaard 2003; Vignuzzi et al. 2006). A similar phenomenon was observed with the mutant CHIKV C483Y, bearing a high-fidelity polymerase that produced less genetic diversity and caused attenuated viral infections in newborn mice and mosquitoes (Coffey et al. 2011). However, in other studies, low-fidelity polymerase mutants of Coxackie virus B3, and CHIKV, also demonstrated attenuated phenotypes and lower viral titers (Gnädig et al. 2012; Rozen-Gagnon et al. 2014). From the aforementioned studies, we might conclude that viral RNA-dependent polymerases are found at a "point of fidelity equilibrium" from which slight changes in any direction show detrimental effects to the viral fitness.

Coronaviruses and Their Peculiar Proofreading Abilities among RNA Viruses

Different works have focused on unraveling the potential proofreading functions of the nsp14 protein during the viral cycle of coronaviruses. Minskaia et al. (2006) showed that nsp14 acts as a 3′-5′ exoribonuclease on both single-stranded and double-stranded RNA, this latter being its preferred substrate. Mutant viruses lacking this protein showed defects during the synthesis of subgenomic and genomic viral RNAs. Working with murine hepatitis virus—another coronavirus—Eckerle et al. (2010) showed that the viral mutation rate was 15- to 20-fold higher in viruses with nsp14 inactive mutants compared with the wild-type viral strains; they later confirmed these findings using the SARS-coronavirus (Eckerle et al. 2010). Smith et al. (2013) showed that in the presence of mutagenic agents such as Ribavirin or 5-FU, the absence of the exonuclease protein significantly increased the number of genomic mutations and also diminished the viral titers within infected cells, demonstrating the role of nsp14 as a proofreading enzyme and a key factor in the coronavirus replication.

The mutation rates calculated for the SARS coronavirus and the mouse hepatitis virus (9.0×10^{-7} and 2.6×10^{-6} mutations per nucleotide per replication cycle (m/n/rc), respectively) were several times lower than those of their corresponding exonuclease-deficient viruses (1.2×10^{-5} and 3.3×10^{-5} m/n/rc, respectively) (Eckerle et al. 2010), and also on the lower end of the range of point mutation rates calculated for most RNA viruses (10^{-3} to 10^{-5} m/s/rc; Lewis et al. 1998). The fact that nsp14 acts as a proofreading enzyme, in a similar way as the exonucleases from the replicative cellular DNA polymerases, might help explain the unique length of the coronaviruses single-stranded linear genome. It is commonly accepted that there is a trade-off between the RNA viruses' genome length and the high mutation rate: for very long RNA genomes, the number of mutations accumulated during each replication cycle would be so elevated that inviable virions would rapidly outnumber the viable ones, leading to a loss of fitness and/or viral extinction. However, Roni and coronaviruses may have overcome this limitation by "acquiring" a correcting enzyme that diminishes the number of mutations. Eckerle et al. (2010) proposed that these viruses might switch the proofreading mechanisms on or off depending on the context, allowing them to rapidly adapt to new environments without losing replicative fidelity.

Other Means to Generate Variability

Even though the high mutation rate caused by the lack of proofreading mechanisms is the main engine in RNA viral evolution, recombination and reassortment have also shown to play a key role. Recombination can be defined as the synthesis of chimeric RNA molecules from two different progeny genomes. Per se, recombination occurs in a single genomic segment. Recombination can be intra-genomic when the two segments come from the same origin, that is, the same infecting virus, or inter-genomic when the two segments come from different origins, that is, different viruses infecting the same cell. In the case of segmented viruses, the packaging within a single virion of genomic segments from different progeny viruses is called reassortment (Chetverin 1999; Pérez-Losada et al. 2015; Simon-Loriere and Holmes 2011).

The main evolutionary consequences of recombination/reassortment in RNA viruses are considered to be an increase in the rate at which beneficial genetic variants are obtained and a more efficient elimination of deleterious mutations (Simon-Loriere and Holmes 2011). However, different theories posit that recombination/reassortment may be a secondary consequence of the selection of other viral characteristics and that physical factors such as genome secondary structure may be driving the rate of this phenomenon in RNA viruses (Pérez-Losada et al. 2015). The presence and the evolutionary role of recombination and reassortment have been demonstrated in the four main groups of RNA viruses; however, the estimated rate of these phenomena among them is highly variable. The virus with the highest recombination rate calculated, as of today, is HIV-1. Each HIV-1 virion contains two copies of the genomic RNA strand (Johnson and Telesnitsky 2010); during the reverse-transcription process, the RT dissociates from the RNA template several times, switching to another RNA strand to use as a template, making a chimeric DNA strand. The in vitro and in vivo estimates of recombination for this retrovirus are of 1.4×10^{-5} to 1.38×10^{-4} recombinations per site per generation (Cromer et al. 2016; Schlub et al. 2014; Shriner et al. 2004). Recombination in HIV-1 has a major impact on the evolutionary history of this virus, and over 60 circulating recombinant forms have been identified in patients around the world. Certain adaptive traits have been associated with circulating recombinant forms, including enhanced biological fitness, increased virulence and pathogenicity, and resistance to antivirals (Vuilleumier and Bonhoeffer 2015).

Thus, from an evolutionary perspective, it appears that RNA viruses benefit largely from random mutations. The close-to-the-edge mutation rate (due to the lack of proofreading mechanisms) results in a very abundant source of potential adaptations against the challenges presented by their hosts, while the recombination/reassortment allows the emergence of new combinations from previously existent mutants. In the following section, the medical challenges posed by the rapid adaptation of RNA viruses will be discussed.

Efforts to Control RNA Viral Diseases: "Fight Diversity with Diversity"

Medical efforts to control viral diseases comprise a variety of strategies at different levels, ranging from the design of drug substances to field surveillance. However, control of RNA viral

diseases has been difficult, because their high adaptive rates enable them to rapidly acquire genetic resistance against traditional control measures (e.g., vaccination or single drug therapies). Thus, modern technologies must also "diversify and evolve" at fast rates to control these rapidly evolving pathogenic agents. Additionally, surveillance and control of a diversity of host populations and reservoirs in the field also plays a key role in overall control measures. Selected examples are provided of modern challenges in drug and treatment designs attempting to overcome the fast adaptive rates of RNA viruses. Also, some field control and surveillance efforts will be presented, as well as those techniques directed to the discovery of new viruses in the wild.

The Pharmaceutical Race against RNA Viruses

The emergence of drug-resistant pathogens is one of the most important medical problems of the last decades (Infectious Diseases Society of America 2011; Spellberg et al. 2008), and RNA viruses are in a position of major concern (Weber et al. 2016). For example, it is known that HIV can acquire significant resistance after just a brief exposure to antiretroviral drugs, especially if they are not combined with additional drugs. In fact, certain high-level resistance mutations to non-nucleoside reverse transcriptase inhibitors can occur with just one point mutation (Lucas 2005).

To inhibit the generation of viral resistance, multidrug therapies (a.k.a. highly active antiretroviral therapy, HAART) emerged as a viable strategy, and soon they became the standard of care for HIV/AIDS patients (Carpenter et al. 1997). Today, with an opportune diagnostic, adequate access to a variety of drugs, and proper adherence to treatment, the expectancy of life of recently infected persons may be similar to that of the HIV-negative population (Nakagawa et al. 2013). Multidrug approaches have also been used against other RNA viruses like the hepatitis C virus (Mizokami et al. 2015) and the influenza virus (Nguyen et al. 2012; Seo et al. 2013). The working mechanism of multidrug therapy is probabilistic in principle: if a viral particle has a random probability to carry one single genetic resistance (i.e., against one single drug), then its probability to carry several combined resistances should decrease geometrically as the number of drug substances increases in the therapeutic regimen. In fact, a simple study by Weverling in 1998 showed that when using five drugs (instead of the standard three-drug regimen), the median time to reach <50 HIV-1 RNA copies/mL was 8 weeks shorter than under the standard—three drug—regimen. However, simply increasing the number of drug substances is not a practical solution, because patients under these treatments can develop severe side effects in the long term (Adams et al. 2004).

Side effects to antiretroviral drugs such as lipodystrophy (i.e., the degeneration of fat in the face, limbs, and upper trunk), hyperlipidemia, and insulin resistance have been reported since the 1990s in patients who received potent HIV protease inhibitors (Carr et al. 1999). In a study regarding the side effects of antiviral therapy against Hepatitis B, Fontana (2009) explains that nucleoside analogues have a potential for inhibiting the human DNA-polymerase-gamma, which is involved in mitochondrial DNA replication; and, when the intracellular mitochondrial DNA numbers decrease, a variety of clinical manifestations may appear, such as neuropathy, myopathy, and lactic acidosis. For example, the nucleoside analogues Adefovir and Tenofovir are associated with a proximal renal tubular toxicity at higher doses. Recent reports suggest that

other comorbidities, such as diabetes mellitus (Dimala et al. 2016), are also possibly associated with HAART therapy.

Secondary effects may result in failure to adhere to the treatment. Thus, despite the effectiveness of multidrug therapies, a lack of adherence to the treatment can still drive the emergence of multi-drug–resistant viral strains by exposing the virus to ineffective doses. Reports of multi-drug–resistant HIV strains appeared since the early literature on HIV; e.g., Larder et al. (1993) reported mutant HIV-1 strains in patients receiving combination therapy with Zidovudine and Didanosine. More recently, multi-drug resistance has also been reported for the 2009 pandemic influenza virus, with viruses sporadically appearing with resistance to neuraminidase inhibitors and adamantanes (Memoli et al. 2010). In a study of multi-resistant hepatitis virus, Yim et al. (2006) mentioned that the generation of multi-drug resistances is facilitated by sequential therapies (i.e., drugs administered sequentially in time), in agreement with previous observations on HIV (Larder et al. 1993). Tamura et al. (2015) presented a revision on multi-drug–resistant viruses as an emergent crisis of the last decades.

Yet some authors argue that the viral genome cannot mutate indefinitely, and mutational resistance must have a cost in terms of reduced replicative fitness for the virus (Hughes and Andersson 2015). Boutwell et al. (2009) demonstrated a reduced replicative capacity of HIV viral strains that coexpressed mutations in the reverse transcriptase against the host's HLA presenting molecule and mutations against antiretroviral drugs. The replicative fitness costs of mutations may explain why, in clinical settings, the most resistant strain is not necessarily the most frequent (Koval et al. 2006). Treatment alternatives based on this principle have been proposed. Domingo and Perales (2016) reviewed the therapeutic approach known as "lethal mutagénesis," which drives viral elimination through excessive mutations. They describe the use of mutagenic nucleotide analogues that have alternate base pairing properties leading to the induction of mutations. Rivabirin was shown to be a mutagenic purine analogue against poliovirus and may have been curing infected patients—inadvertently— through the mechanism of lethal mutagenesis. Rivabirin has also been suggested as a mutagenic agent against Hepatitis C virus (Cuevas et al. 2009). The potential use of lethal mutagenesis may be restricted, for example, when used against coronaviruses that possess proofreading enzymatic capabilities. The lethal mutagenesis approach needs further development, especially regarding the search of nontoxic mutagens, but it promises to be a viable antiviral strategy.

Interestingly, RNA viruses may even be able to counteract a lowered replication fitness (due to drug-resistance mutations) when additional mutations compensate for such loss of fitness. Recent investigations reported that fitness reductions from resistance mutations in the HIV protease can be "rescued" by other mutations in the vicinity of the viral Gag proteolytic cleavage sites, which leads to improved processing of Gag by the highly mutated protease (Kožíšek et al. 2012). Thus, these additional mutations confer a "compensatory mechanism" for the reduced fitness of an enzyme (Hughes and Andersson 2015). Compensatory mutations have also been reported for influenza A (H1N1) viruses, which compensated for deficiencies in viral function and enabled the virus to acquire the H275Y NA resistance mutation without loss of fitness, "resulting in its rapid global spread" (Hurt 2014).

The extraordinary evolutionary capabilities of RNA viruses have stimulated the development of an entire diversity of pharmaceutical alternative strategies. Other recent strategies also

include the use of modern molecular techniques such as RNA silencing, which consists in the use of RNA sequences (siRNA) complementary to specific messenger RNA sequences; base pairing of these complementary sequences drives the formation of double-stranded RNA and induces the degradation of the original messenger RNA. For example, Jacque et al. (2002) directed siRNAs against several regions of the HIV-1 genome and demonstrated a reduction of >95% in viral infection. Also, the use of siRNAs significantly reduced lung influenza virus titers in infected mice and protected them from lethal challenge (Tompkins et al. 2004). These therapies are, however, on their early stages of development and still have some difficulties. Chen et al. (2008) stated that a main obstacle to the use of RNA interference (for the treatment of chronic HBV infection) has been the lack of safe and effective delivery of the siRNA trigger molecule. In this regard, some authors have developed special delivery systems; for example, Rozema et al. (2007) described a polymer-based system, named Dynamic PolyConjugate, for the targeted delivery of siRNA to hepatocytes.

Modern techniques in vaccine development have also introduced interesting evolutionary control methods, for example, the manipulation of codon pair biases (Coleman et al. 2008). On the basis of codon degeneracy in the genetic code (i.e., the existence of synonymous codons that translate into the same amino acid) and the fact that some synonymous codons are translated less frequently than others, Coleman and collaborators (2008) developed an attenuated poliovirus strain. Their "customized" polioviruses contained genetic sequences with hundreds of under-translated synonymous codons. The replication process of these genetic sequences within a host cell (in mice) would therefore be less efficient than that of the wild-type virus. The authors reported that attenuated strains provided protective immunity to mice after challenge.

Although treatments against poliovirus, influenza, hepatitis C, and HIV infections have achieved important advances in the last decades, specific drugs are yet to be designed to control several of the remaining—and highly pathogenic—RNA viruses (Bray 2008).

Is It Possible to Target Unknown or Recently Emerged RNA Viruses?

In some cases, when confronting a "new" virus (or one for which specific drugs have not yet been designed), the best option is to use known drug substances against other RNA viruses. For example, Lamivudine (a nucleoside-analog reverse-transcriptase inhibitor) is used as an antiretroviral drug against hepatitis B and HIV, but during the last EBOV outbreak, it was reported that the treatment with Lamivudine early in the infection resulted in the cure of 13 of 15 patients (Azango 2014). In investigating the tridimensional structure of the EBOV RNA polymerase, Jácome et al. (2015) indicate that certain conserved subdomains in the architecture of these proteins may help to explain the broad antiviral activity of certain nucleotide analogues, even against different types of RNA viruses, including ss (−) and ss(+) RNA genomes. Moreover, Brincidofovir has antiviral activity against both DNA and RNA viruses. They concluded that, while specific drugs against EBOV are yet to be designed, other drugs aimed at the active site of other polymerases might interfere with the functionality of the EBOV RNA polymerase, albeit with less specificity. These considerations highlight the importance of phylogenetic studies of newly discovered viruses; such studies may help to direct emergency therapeutic actions before the design of specific antiviral drugs. Interestingly, although the common ancestry of EBOV and retroviruses is not yet clear, it is known that the L protein of EBOV is homologous to the reverse transcriptase of HIV-1 (Jácome et al. 2015). In both proteins, the active site (i.e., the palm subdomain) is highly conserved; ergo, the inhibiting effects of drugs can be expected against both proteins. In this same regard, the development of heterologous vaccines within filoviruses has also been explored: Warfield et al. (2015) demonstrated protective efficacy using EBOV virus-like particles against Taï Forest virus, both within the *Ebolavirus* genus. Although there are not "universal" drugs against all RNA viruses, it is possible that certain substances against similar viruses may serve a purpose for preliminary control in an emergency situation.

Unfortunately, relative phylogenetic closeness is not always a useful aid in vaccine development; for example, despite that the pathogenesis of SARS had been well characterized, much of this knowledge was not applicable to the MERS virus (Maslow 2017). It was known that SARS infects cells by binding to an enzyme widely expressed in mammals (i.e., the angiotensin converting enzyme 2; Wong et al. 2004). Because of this, murine and other mammalian animals were readily used as models to test candidate vaccines against SARS. On the other hand, however, the MERS virus binds to the cell surface dipeptidylpeptidase 4, in which the receptor binding domain differs between susceptible and nonsusceptible species (Li et al. 2003). MERS is restricted to primates, camelids, and bats. For this reason, rodents were not useful models, whereas camels, alpacas, and primates did develop infection (Crameri et al. 2016) but are expensive models for vaccine testing. These kind of difficulties can delay the development of vaccines against emerging diseases. Also, the lack of data on immunogenicity, dosing, and safety can hinder progress (Røttingen et al. 2017). Faster vaccine developments must be a priority when responding to outbreak emergencies.

The EBOV outbreak in Africa also boosted changes to the classical paradigm of vaccine development against emerging infectious diseases. These alterations included, for example, the advancement of studies into Phase II/III while Phase I was still being completed. Also, novel clinical trial designs emerged, in particular, the "ring vaccination strategy" (Maslow 2017). This strategy uses a staged approach in which direct contacts of patients infected with EBOV are grouped into "rings," and each ring is randomized into early (immediate) versus late (delayed by 21 days) inoculation. This design has been used to test the efficacy of the rVSV-vectored vaccine expressing Ebola surface glycoprotein. Henao-Restrepo et al. (2015) reported no cases of EBOV disease in the immediate vaccination group (4123 people), while 16 cases were found in the delayed vaccination group (3528 people). According to Maslow (2017), vaccines for emerging infectious diseases, such as EBOV, MERS, and ZIKV, represent a unique paradigm to standard vaccine development principles.

Additional efforts to control and prevent viral diseases extend from the laboratory and the clinic to wildlife surveillance. In the following section, a few examples will be given of modern field survey techniques and strategies to monitor potential viral threats.

Field Survey Strategies

Since nearly 80% of viral diseases that infect humans are zoonotic (Morse et al. 2012), field surveys of RNA viruses must be conducted by monitoring their domestic and/or wildlife reservoir populations. Table 2 shows a few examples of zoonotic

Table 2 Examples of zoonotic RNA viruses, their known or suspected reservoirs, and reported routes of transmission

Group	Virus	Known or suspected reservoir(s)	Reported route(s) of zoonotic transmission	Reference
(+) Single-stranded	Foot-and-mouth disease virus	Wild and domestic bovines	Shared water sources	Miguel et al. (2013)
	Chikungunya virus	Nonhuman primates	Mosquito vectors	Althouse et al. (2016)
	West Nile virus	Birds	Mosquito vectors	Zeller and Schuffenecker (2004)
	SARS coronavirus	Bats	Direct contact during wildlife trading/ butchering, respiratory droplet transmission	Fong (2017), Hampton (2005)
(−) Single-stranded	Influenza A virus	Birds/swine	Aerosols and direct contact with reservoirs	Varble et al. (2014)
	Ebola virus	Fruit and insectivorous bats	Direct contact (hunting or butchering)	Saéz et al. (2015)
	Nipah virus	Fruit bats	Contaminated fruit	Luby et al. (2006)
Double-stranded	Colorado tick fever virus	Squirrels and chipmunks	Tick vector (*Dermacentor andersoni*)	Stahl et al. (2011)
	Banna virus	Unknown (isolated from mammals)	Mosquito vectors	Mohd Jaafar and Attoui (2009)
Reverse transcribing	Primate T-lymphotropic viruses	Primates	Living in close contact with infected nonhuman primates	Richard et al. (2016)

RNA viruses, their known or suspected reservoirs, and their routes of zoonotic transmission. A reservoir can be understood as a species (or taxonomic group) in which the pathogen can be permanently maintained and from which infection may be transmitted to another species (definition modified from Haydon et al. 2002). Knowing the ecology of reservoir species and main routes of interspecies transmission is central to any preventive campaign.

In particular, large and dense reservoir populations may be predictive of a large pathogen load and virulence (Anderson et al. 1992). Because of this, certain species of bats (Calisher et al. 2006) and bird species (Cui et al. 2014) are being increasingly recognized as major reservoirs for several viruses. Remarkably, it has been reported that bats can serve as reservoir hosts of a greater viral diversity than other host species, for example, rodents (Luis et al. 2013; O'Shea et al. 2014). Such reservoir capabilities of bats may arise, firstly, because of their roosting behavior in large and dense congregations that greatly promote transmission and, secondly, because of their flying capabilities and ample home ranges that potentially allow them to translocate viral strains across large geographic regions. Drexler et al. (2012) suggested a predominance of host switches from bats to other mammals and birds. In fact, they placed bats as tentative ancestral hosts to both the major Paramyxoviridae subfamilies (Paramyxovirinae and Pneumovirinae). Paramyxoviridae species are responsible for some significant human and domestic animal viral diseases, such as measles, distemper, mumps, parainfluenza, and Newcastle disease.

Other relevant animal species in the transmission cycle of viral diseases include vectors, such as the mosquito *Aedes aegypti* and *A. albopictus*, which can spread DENV, CHIKV, ZIKV, and Yellow Fever virus (YFV) (Barbazan et al. 2008; Lenhart et al. 2013). Efforts to monitor these species also include the use of modern remote sensing technologies, such as the use of satellite imagery and climatic data to predict their potential distribution (Quattrochi et al. 2014).

Field surveillance strategies must also incorporate molecular techniques that allow the identification of novel viruses, instead of monitoring only a few well-known viral species. Modern sequencing techniques (e.g., shotgun sequencing) have allowed the discovery of novel plant and animal viruses (Al Rwahnih et al. 2009); for example, in the work of Drexler et al. (2012), samples from 119 bat and rodent species allowed the identification of 66 new paramyxoviruses. In 2010, Li et al. (2010) proposed an interesting field survey strategy that involved sequencing all viral genomes contained in bat guano. In their results, a total of 390,000 sequence reads from bat guano in California and Texas allowed them to identify viruses infecting insects (reflecting the diet of insectivorous bats), plants, and fungi (possibly reflecting the diet of ingested insects) and also viral sequences that infected mammals (the latter included the families Parvoviridae, Circoviridae, Picornaviridae, Adenoviridae, Poxviridae, Astroviridae, and Coronaviridae). Hence, the peculiar habits of wildlife populations are not only useful for studying the viruses they carry and transmit, but they may also be useful for assessing the viral biodiversity in their ecosystems.

However, these modern sequencing techniques can be economically restrictive in certain developing countries. Unfortunately, the most susceptible environments to infectious diseases in the tropics also coincide with significant economical limitations for local health authorities. Thus, it is essential to find alternatives that are effective and economically accessible. An interesting example can be found in the work of Villinger et al. (2016), in which a low-cost technique was used (i.e., High Resolution Melting RT-PCR). This technique evaluates viral diversity through the dissociation curves of DNA versus temperature instead of using costly sequencing techniques. Also, to identify potentially new viruses, they used degenerate primers with a specific design that preferentially targeted RNA viral genomes over the host's RNA. Villinger et al. (2016) reported results comparable to gold standards and described the presence of a clade of hemorrhagic fever arboviruses that had not been previously isolated in Kenia. These low-cost strategies may represent a feasible approach in the low-budget scenarios frequently found at those regions exposed to infectious pathogens.

Field surveillance of wildlife populations with accessible molecular techniques may help to reveal viral threats; still, determining their emergence or reemergence in space and time

is difficult. Predictive modelling has a great potential for prevention and control strategies (Eisen and Eisen 2011; Fischer et al. 2014; Gao et al. 2016; Wu and Cowling 2011). In particular, remotely sensed environmental factors allow predicting vector/reservoir abundance for large and continuous geographic regions. As an example, through the combination of remotely sensed environmental data and point data of mosquito abundance (from light trap collections), Diuk-Wasser et al. (2006) developed a logistic model for low and high abundance of mosquito vectors of the West Nile virus in Connecticut, United States. Their models predicted high abundances of *Culex pipien* in nonforested areas, while surface water and distance to estuaries predicted high abundances of *C. Salinariu*, also, surface water and grasslands/agriculture predicted for *Aedes vexans* and, finally, seasonal difference in the normalized difference vegetation index and distance to palustrine habitats predicted for *Culiseta melanura*. An interesting model by Peterson et al. (2003) suggested that spread patterns of the West Nile virus were best explained when including migratory birds as critical long-distance transport agents. Other mathematical models can assess, for example, the population effect of vaccination in domestic animals to prevent the development of zoonotic diseases. Reynolds et al. (2014) found that, after an influenza outbreak in a swine breeding herd, their models predicted a persistently high level of infectious piglets, which was robust even after changes in transmission rates and farm size. Their models also predicted that vaccination did not eliminate influenza after an outbreak.

In general, the monitoring, prediction, and control of RNA viral diseases have proven to be difficult tasks, and every effort is crucial, from the design of novel drug therapies to the discovery of potential new threats in the wild. In addition, the ecological knowledge of how zoonotic outbreaks develop is also fundamental for the prevention and control of RNA viral diseases.

Natural Histories of Interspecies Transmissions

Known examples of interspecies pandemics are strongly related to global land use changes. The invasion of natural ecosystems and the growth of dense human settlements—as well as the growth of global trade and mobility—are driving increased rates of interspecies contacts and the interchange of parasites and pathogens that can develop into global pandemics. These phenomena comprise intricate networks with several actors, from wildlife species to domestic animals and the globally interacting human population. In this section, we will review some examples that illustrate the natural history of zoonotic outbreaks of RNA viruses.

Influenza Virus Type A: A Story of Emergence and Reemergence

"Major influenza epidemics have apparently occurred since at least the Middle Ages" (Taubenberger and Morens 2010), with well-registered pandemics in 1889, 1918, 1957, 1968, 1977, and 2009. Since 2013, there have been several outbreaks of Influenza viral strains. In North America, the spread of highly pathogenic avian influenza H5 viruses has been reported, and zoonotic H10N8 and H5N6 infections were detected; China also presents ongoing H7N9 infections; and, in the Middle East, H5N1 zoonotic infections continue to occur (Joseph et al. 2016).

It is well known that wild aquatic birds are natural reservoirs of IAV subtypes H1-H16 (Joseph et al. 2016), but also subtypes H17 and H18 were found in bat species (Tong et al. 2012).

Viral strains usually circulate endemically within their natural reservoirs (i.e., enzootically); however, continuous interspecies contact may facilitate the "spill-over" of a viral strain towards other species (i.e., an epizootic event). Therefore, in addition to their wildlife reservoirs, influenza viruses can infect a range of host species that include domestic animals and humans (Webster et al. 1992). The colonization of a new host species may require viral diversification strategies to escape the host's immune system, and IAV's ability for gene recombination and its rapid genetic and antigenic evolution enables it to readily adapt to new immunogenic environments, also making vaccination efforts difficult in humans and domestic animals (Webster et al. 2014). Wild migratory waterfowl and waterbirds are the source of viral subtypes antigenically novel to humans (Obenauer et al. 2006). It is now known that the introduction of influenza strains with a novel subtype into human circulation caused both the 1957 and 1968 pandemics due to antigenic shift (Taubenberger and Morens 2010). Antigenic shift refers to a phenotypic change of viral surface antigens due to genetic recombination; novel combinations can be different enough (from the original strains) to avoid the immune memory of previously exposed individuals.

Influenza strains found in domestic animals, such as pigs (Kida et al. 1994) and dogs (Song et al. 2008), usually represent a significant threat to human health, where domestic animals act as intermediate hosts in which reassortment between avian and human viruses occur. In fact, molecular analyses show that most IAV strains of subtypes H1 and H3 found in humans were closely related to swine IAV strains (Joseph et al. 2016). Interestingly, ferrets have also been identified as hosts of the recently emerged H7N9 strain (Zhu et al. 2013). Although direct transmission of IAV from wild birds to humans may be rare, there is at least one laboratory-confirmed report of H5N1 contracted through interaction with dead wild swans in Azerbaijan (Gilsdorf et al. 2005). Also, serological evidence of exposure to H5A1 has also been found in Alaskan hunters who interact with dead wild avian specimens (Reed et al. 2014). The recent detection of a 1918-like H1N1 avian virus in wildlife populations has raised concerns about the potential reemergence of a 1918-like pandemic (Watanabe et al. 2014). Joseph et al. (2016) emphasized that the increasing human intrusion into wildlife habitats increases the risks for the emergence and reemergence of IAV strains.

CHIKV: The Story of a Recent Vector-Borne Zoonotic Disease

The mosquito-borne CHIKV was first discovered in present day Tanzania during the 1952–1953 outbreak and has since spread to Africa, Asia, Europe, the South Pacific, and—most recently—to the Americas (Weaver and Forrester 2015). The enzootic transmission cycles of CHIKV in sub-Saharan Africa consist of forest-dwelling mosquito vectors and nonhuman primates as host species (i.e., the sylvatic cycle) (Althouse et al. 2016; Tsetsarkin et al. 2016; Weaver and Forrester 2015). The urban CHIKV cycle affecting humans presumably originated when people living near African forests became infected by bites from wildlife mosquito species such as *Aedes furcifer*. *A. furcifer* mosquitoes may occasionally occupy urban niches within villages settled in the vicinity of forests (Diallo et al. 2012). However, a different mosquito species, *Aedes aegyptii*, better adapted to artificial water containers as oviposition sites and to human blood as its source of nutrients, became globally widespread with human migrations (Powell and Tabachnick 2013).

Similarly although more recently, the mosquito *A. albopictus* has had a history of global expansion facilitated by human migration and trade. As a matter of fact, the introduction of *A. albopictus* into the United States was reported since the 1980s (Reiter and Sprenger 1987), for which the intercontinental shipment of used tires was, at least, partially responsible. CHIKV epidemics apparently arose when people infected in spillover events from enzootic CHIKV reached a location where populations of domestic mosquitoes and their contact with people were adequate to initiate inter-human transmission (i.e., the urban transmission cycle) (Tsetsarkin et al. 2016).

Weaver (2013) enlisted some preventive strategies against arthropod-borne viruses. The author indicates that arthropod-borne RNA viruses such as DENV, YFV, and CHIKV presumably spill from the sylvatic cycle by infecting people living in the vicinity of natural areas who may ultimately transport the virus to the urban cycle. Thus, viable prevention strategies against spillover events are to reduce the exposure of these human populations to sylvatic vectors and/or to reduce their susceptibility through vaccination. Simple solutions for reduced exposure include the use of bed nets that offer some protection against mosquito bites. In turn, the prospects for developing a vaccine against CHIKV are in fact promising due to the limited antigenic variation of the virus (Weaver et al. 2012). Vaccination campaigns should be preferentially targeted to those people in closer contact with the sylvatic cycles. Weaver et al. (2012) stress the importance of prioritizing the development of vaccines against these vector-borne viruses.

SARS and the Wildlife Trade

In 2003, the SARS coronavirus spread over 29 countries in 5 continents, leaving 774 deaths and an estimated cost of $16.8 billion in China's tourism profits (Greatorex et al. 2016). In the literature, it is suggested that increasing international wildlife trade between countries such as China, Vietnam, and Lao People's Democratic Republic may have played an important role in the 2003 SARS outbreak (Bell et al. 2004; Greatorex et al. 2016). According to Greatorex and collaborators, wildlife meat has existed as a dietary component in Lao for many generations, but such practices had remained limited to local subsistence consumption. However, after the economic opening of the country in the early 1980s, wildlife trade "gained momentum," establishing the onset for the further development of an epidemic. In their observational study, at markets in Lao, Greatorex et al. (2016) identified four relevant risk factors in the zoonotic transmission of wildlife diseases:

1. Wildlife-human contact. In high-volume markets, the authors found an average daily count of alive (or fresh dead) animals ranging from 22 to 931 animals per day being handled in each market.
2. Trade of animals potentially carrying zoonotic diseases. The authors observed the trade of 12 families of mammals: Muridae (rat species), Suidae (wild pig), Pteropodidae (fruit bats), Sciuridae (tree and flying squirrels), Cervidae (muntjac, sambar), Leporidae (hare), Felidae (leopard cat), Rhinolophidae (insectivorous bats), Viveridae (civets), Herpestidae (mongoose), Hystricidae (porcupine), and Lorisidae (loris), capable of hosting 36 significant zoonoses (including rabies, SARS, leptospirosis, and the Mycobacterium tuberculosis complex).
3. Poor biosafety. Market personnel showed risky behaviors for food contamination, such as lack of hand washing and cleaning of tables, the practice of selling wildlife alongside other

fresh products, and poor market cleanliness in general. These factors represent risks for direct human infection.
4. Potential for human spread of a disease from markets to wider populations. In their study, the majority of high-volume markets were located within large towns or on main roads, facilitating contact with large human settlements. Also, they were able to observe foreigner visitors and license plates from other countries at the markets, suggesting a potential for international spread.

Activities in wildlife markets are, therefore, a potential factor in the onset of zoonotic outbreaks. The abovementioned characteristics must be assessed when studying the development of epidemics or when directing efforts to prevent them. Also, according to Bell et al. (2004), a major lesson from the SARS outbreak is that newly emergent zoonotic diseases occur in parallel with biodiversity crises of wildlife overexploitation, through the increase human activities and commercial demand.

What Have We Learned?

According to Holmes (2013), there are some evolutionary and ecological generalities that may allow large-scale predictions of interspecific transmission. A well-established rule is that pathogens are more likely to "jump" between phylogenetically related hosts. For example, Streicker et al. (2010) demonstrated that frequencies of interspecies transmission of the rabies virus decreased with increasing phylogenetic distance between bat host species. Yet such phylogenetic barrier appears to be less significant for viruses than it is for other pathogens. Davies and Pedersen (2008) studied different pathogen communities (protozoans, helminthes, and viruses) in primates and humans and observed that—for viruses—host phylogenetic distance is less important than geography in explaining pathogen community similarity between hosts. They suggested that geographical overlap between neighboring hosts is more relevant due to the rapid evolution of viral lineages allowing them to "jump" hosts across larger evolutionary distances. Moreover, Holmes (2009) highlights that RNA viruses jump species boundaries more often than DNA viruses, and this likely arises from their differing rates of evolutionary change.

As for the ecological "rules" that explain patterns and processes of viral emergence, it is likely that most instances of viral emergence have their roots in ecological perturbation. In this case, quantitative models that account for the localization of human disturbance will undoubtedly help predict future interspecies transmission events. For example, land use gradients may modify the dispersion and genetic evolution of viruses (Zirkel et al. 2011). Zirkel et al. (2011) showed that, in a gradient from tropical rainforest to agricultural land use, the genetic diversity of a newly discovered Nidovirus (Cavally virus) decreased, while its prevalence increased in the mosquito population along the process of spreading into disturbed habitats. In a study with ranaviruses (double-stranded DNA viruses), Price et al. (2016) revealed a significant trend for elevated rates of outbreaks in localities with higher human population density. These patterns were likely explained by anthropogenic translocation of viruses from other countries that stimulated the spread of novel viral strains in densely populated areas (Price et al. 2016). On the other hand, it has been proposed that disease risk and interspecies transmission is lower where ecosystems and trophic chains remain conserved (Ostfeld and Holt 2004).

Interspecies transmission alone, however, is not the only factor to consider for potential pandemics, because a majority

of epizootic events result only in "dead-end spillover" infections (i.e., in which the virus cannot establish onward transmission in the human population). Yet some pandemics, for example EBOV (Gire et al. 2014), SARS, and HIV (Ostfeld and Holt 2004), were capable of establishing transmission networks among human hosts; but these are exceptions to the common zoonotic pattern, in which humans can only acquire an infection from animal reservoirs. Geoghegan et al. (2016) presented a very interesting analysis on the virological features that may predict adequacy to human emergence. The authors used a multivariate approach to assess the best predictors for human-to-human transmission. They determined that viruses that induce low host mortality, establish long-term chronic infections, and that are nonsegmented, nonenveloped, and not transmitted by vectors were more likely to be transmissible between humans. In regard to the low transmissibility of vector-borne viruses, Coffey et al. (2008) explained that interspecies transfers in arboviruses may be constrained by their alternating infection of dissimilar hosts, where optimal reproduction in one host may involve a fitness tradeoff for the other. Additionally, in their multivariate study, Geoghegan et al. (2016) found that genomic variables had lower predictive power than the aforesaid virological features. In fact, Holmes (2013) had suggested previously that viral genetic studies would be informative only if it were possible to associate such genetic information with specific phenotypes. Any virus identified with the ability to change host species and successfully establish transmission within the human population should therefore be of special concern. Recent evidence has been published for the sexual transmission of ZIKV (D'Ortenzio et al. 2016; Musso et al. 2015), which poses it as a major public health emergency around the globe. Through mathematical modeling, Gao et al. (2016) suggest that sexual transmission of ZIKV can increase the risk of infection and size of the epidemic as well as prolong the outbreaks.

Regarding the risk of human-to-human transmission, Woolhouse et al. (2016) classified viruses in four levels according to their basic reproduction number or rate of transmission between humans (R_0, defined as the average number of secondary cases generated by each single primary case). Level 1 viruses would be those without current ability to infect humans, present only in wildlife populations, but that may eventually "jump" to humans (as it was observed with SARS, EBOV, and MERS-CoV); of special concern are those found in primate species, but also in bats and birds (R_0 = undefined, as the primary case is inexistent). Level 2 viruses are those that infect humans but do not spread in the human population (e.g., Rabies virus; $R_0 = 0$), yet these viruses may eventually acquire the genetic ability to spread amongst humans. It is hypothesized that the HIV-1 M lineage emerged from the only strain of simian immunodeficiency viruses capable to overcome a key host restriction (i.e., the human tetherin) (Sharp and Hahn 2011). Level 3 viruses are those that may occasionally transmit between humans ($0 < R_0 < 1$); for example, a novel Rhabdovirus, the Bas-Congo virus, has shown transmissibility in humans (Woolhouse et al. 2016). Finally, Level 4 viruses are those that spread epidemically amongst humans ($R_0 > 1$); interestingly, arboviral, Level 4 viruses (ZIKV, CHIKV, YFV, DENV) indicate that high-level spread in human populations is linked to carriage by anthropophillic vectors.

In general, risk assessment of potential zoonotic viral threats requires the combination of genetic, phenotypic, and epidemiological information of viruses along with that of the ecology of reservoirs/vectors and the expansion of human activities affecting natural ecosystems.

The Immediate Future of RNA Viral Threats

Future epidemics are difficult to predict, but the opinion of experts may shed some light on what can be expected for the years to come. Rodríguez-Morales et al. (2016) asked whether a new viral threat could be expected in the Americas for 2017, following the arrival of CHIKV in 2013 and Zika in 2015. Yet instead of concern about intercontinental threats, the authors point to an "insider," the local Mayaro virus (MAYV). MAYV is an arbovirus with a sylvatic transmission cycle similar to that of YFV and CHIKV. Moreover, per these authors, the symptoms of the MAYV infection overlap and can be easily confused with those of CHIKV, ZIKV, and DENV, posing a significant diagnostic challenge and a novel threat as the next potential emerging pathogen in the Americas. In Asia, new emerging viruses are also being described, such as the Banna virus that "shows rapid evolutionary rates and a potential for introducing into non-endemic areas" (Liu et al. 2016).

On the other hand, long-known viral diseases should not be overlooked, as they can still represent a threat despite medical efforts. Even though more than 650 million vaccine doses against YFV have been distributed in the past 75 years, in December 2015, a YFV outbreak was identified in Angola. By May 20, 2016, a total of 2420 suspected cases had been reported, including 298 deaths. Thus, a committee convened by the World Health Organization on May 19, 2016 decided that the current epidemic is a "serious public health concern" (Barrett 2016). Moreover, according to Barrett, nearly 6 million YFV doses are reserved for emergencies. However, these reserves may not be sufficient to meet the demand of large outbreaks, especially in areas where YFV has gone decades without an urban outbreak, as it was the case of Angola in 2015. The current plan of action (May 2016) of the WHO Research and Development Blueprint includes other viral diseases to be urgently addressed and necessitating further action as soon as possible (i.e., Crimean Congo hemorrhagic fever virus, EBOV, Marburg virus, SARS-CoV, MERS-CoV, Lassa fever virus, Nipah virus, Rift Valley fever virus, CHIKV) (in Røttingen et al. 2017).

Constant monitoring of RNA viral diseases and preventive pharmaceutical production are therefore crucial for timely detection and intervention to avoid severe outbreaks in the future. In this regard, the work of Hotez (2017) stresses the need for economic and political innovations that run parallel with scientific efforts against emergent and neglected diseases. Hotez proposes a combination of global funds, such as the Coalition for Epidemic Preparedness Innovations, alongside national funds from wealthy countries (G20 nations), which, paradoxically and according to Hotez's work, account for most of the world's poverty-related illnesses; this in order to secure sufficient funding for the timely development of adequate monitoring and pharmaceutical response.

Finally, the control of certain RNA viral diseases is also problematic for social reasons; for example, the global control of HIV is currently below expected results, because reaching vulnerable communities is reportedly challenging due to the social stigma against HIV-infected people. In certain circumstances, HIV-positive patients may reject treatment for fear of being seen at the local clinics (Gilbert and Walker 2010) or being targets of discrimination from healthcare providers (Chan et al. 2015; Katz et al. 2013; Stringer et al. 2016) despite the expansion of access to drug therapy. Additionally, the global economic crises have driven general budget cuts in HIV control efforts. These difficulties potentially explain an alarming deceleration of the annual decline of new HIV infections in the past years, with some countries (including Egypt, Mexico, Russia, and the Philippines) having slightly increased rates of new infections

([Steel 2016](#)). Unfortunately, disease control is ultimately limited by economic and social constraints. Thus, the design of pharmaceutical substances and surveying techniques must also cope with the feasibility of their implementation at the population level, which involves a complex network of educators and policy and decision makers. It must be acknowledged that it is not only human activities in natural ecosystems but also current budgets, policies, and social knowledge—at the local and global levels—that can determine the progress of an epidemic.

General Conclusion

Pathogenic RNA viruses are one of the most important groups of pathogens involved in zoonotic transmission events and are a challenge for global disease control. Not only the unknown viral species in the wild, but also those that have been known for decades—or even centuries—still represent a continuous problem to human and animal health. Their biological diversity and rapid adaptive rates have proven to be difficult to overcome and have stimulated the continuous development of pharmaceutical and medical technology. The technological development specifically designed for the survey and control of RNA viruses must therefore be a research priority. Additionally, the continuous monitoring of viral genetics and phenotypes in wildlife reservoirs is also crucial, as reservoirs may be a constant source of novel pathogenic material for humans. Human activities and policies are possibly the best predictors of the extent and severity of future epidemics. Perhaps the best strategy against RNA viral diseases is to design preventive survey programs that evaluate the most vulnerable sectors and geographic regions. These programs will aid in developing plans that guarantee the existence of sufficient and adequate access to treatment. Finally, conservation policies that control the disturbance of natural ecosystems are also essential.

Acknowledgements

References

Adams B, Banks H, Kwon H-D, Tran HT. 2004. Dynamic multi-drug therapies for HIV: Optimal and STI control approaches. Math Biosci Eng 1(2):223–241.

Afreen N, Naqvi IH, Broor S, Ahmed A, Kazim SN, Dohare R, Kumar M, Parveen S. 2016. Evolutionary analysis of Dengue serotype 2 viruses using phylogenetic and Bayesian methods from New Delhi, India. PLoS Negl Trop Dis 10(3):e0004511.

Al Rwahnih M, Daubert S, Golino D, Rowhani A. 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. Virology 387(2):395–401.

Althouse BM, Guerbois M, Cummings DA, Diop O, Faye O, Faye A, Diallo D, Sadio BD, Sow A, Faye O. 2016. Monkey in the middle: Monkeys serve as amplification hosts but not reservoir hosts of sylvatic Chikungunya virus. bioRxiv:079046.

Álvarez M, Menéndez-Arias L. 2014. Temperature effects on the fidelity of a thermostable HIV-1 reverse transcriptase. FEBS J 281(1):342–351.

Anderson RM, May RM, Anderson B. 1992. Infectious diseases of humans: dynamics and control. Oxford university press, Oxford, UK.

Añez G, Grinev A, Chancey C, Ball C, Akolkar N, Land KJ, Winkelman V, Stramer SL, Kramer LD, Rios M. 2013. Evolutionary dynamics of West Nile Virus in the United States, 1999–2011: Phylogeny, selection pressure, and evolutionary time-scale analysis. PLoS Negl Trop Dis 7:e2245.

Azango M. 2014. Liberian doctor defends 3–5 days Ebola treatment with HIV drug. Available online (http://allafrica.com/stories/201409292050.html), accessed on 20 November 2016.

Barbazan P, Tuntaprasart W, Souris M, Demoraes F, Nitatpattana N, Boonyuan W, Gonzalez J-P. 2008. Assessment of a new strategy, based on Aedes aegypti (L.) pupal productivity, for the surveillance and control of dengue transmission in Thailand. Ann Trop Med Parasitol 102:161–171.

Barrett AD. 2016. Yellow Fever in Angola and beyond—the problem of vaccine supply and demand. N Engl J Med 375(4): 301–303.

Bell D, Roberton S, Hunter PR. 2004. Animal origins of SARS coronavirus: Possible links with the international trade in small carnivores. Philos Trans R Soc Lond B Biol Sci 359 (1447):1107–1114.

Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V, Gubler DJ, McMillan WO. 2003. Selection-driven evolution of emergent dengue virus. Mol Biol Evol 20(10): 1650–1658.

Binder S, Levitt AM, Sacks JJ, Hughes JM. 1999. Emerging infectious diseases: Public health issues for the 21st century. Science 284(5418):1311–1313.

Bopp SE, Manary M, Bright AT, Johnston GL, Dharia NV, Luna FL, McCormack S, Plouffe D, McNamara CW, Walker JR, Fidock DA. 2013. Mitotic evolution of Plasmodium falciparum shows a stable core genome but recombination in antigen families. PLoS Genet 9(2):e1003293.

Boutwell CL, Rowley CF, Essex M. 2009. Reduced viral replication capacity of human immunodeficiency virus type 1 subtype C caused by cytotoxic-T-lymphocyte escape mutations in HLA-B57 epitopes of capsid protein. J Virol 83(6): 2460–2468.

Bray M. 2008. Highly pathogenic RNA viral infections: Challenges for antiviral research. Antiviral Res 78(1):1–8.

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. 2006. Bats: Important reservoir hosts of emerging viruses. Clin Microbiol Rev 19(3):531–545.

Carpenter CC, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, Montaner JS, Richman DD, Saag MS, Schooley RT. 1997. Antiretroviral therapy for HIV infection in 1997. J Am Med Assoc 277(24):1962–1969.

Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. 1999. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: A cohort study. Lancet 353(9170):2093–2099.

Castro C, Arnold JJ, Cameron CE. 2005. Incorporation fidelity of the viral RNA-dependent RNA polymerase: A kinetic, thermodynamic and structural perspective. Virus Res 107(2): 141–149.

Cattoli G, Fusaro A, Monne I, Coven F, Joannis T, El-Hamid HS, Hussein AA, Cornelius C, Amarin NM, Mancin M, Holmes EC, Capua I. 2011. Evidence for differing evolutionary dynamics of A/H5N1 viruses among countries applying or not applying avian influenza vaccination in poultry. Vaccine 29:9368–9375.

Chan BT, Weiser SD, Boum Y, Siedner MJ, Mocello AR, Haberer JE, Hunt PW, Martin JN, Mayer KH, Bangsberg DR. 2015.

Persistent HIV-related stigma in rural Uganda during a period of increasing HIV incidence despite treatment expansion. AIDS 29(1):83.

Chen Y, Cheng G, Mahato RI. 2008. RNAi for treating hepatitis B viral infection. Pharm Res 25(1):72–86.

Chen Y, Sharp PM, Fowkes M, Kocher O, Joseph JT, Koralnik IJ. 2004. Analysis of 15 novel full-length BK virus sequences from three individuals: evidence of a high intra-strain genetic diversity. J Gen Virol 85:2651–2663.

Cherian SS, Walimbe AM, Jadhav SM, Gandhe SS, Hundekar SL, Mishra AC, Arankalle VA. 2009. Evolutionary rates and timescale comparison of Chikungunya viruses inferred from the whole genome/E1 gene with special reference to the 2005–07 outbreak in the Indian subcontinent. Infect Genet Evol 9(1):16–23.

Chetverin AB. 1999. The puzzle of RNA recombination. FEBS Lett 460(1):1–5.

Coffey LL, Beeharry Y, Bordería AV, Blanc H, Vignuzzi M. 2011. Arbovirus high fidelity variant loses fitness in mosquitoes and mice. Proc Nat Acad Sci USA 108(38):16038–16043.

Coffey LL, Vasilakis N, Brault AC, Powers AM, Tripet F, Weaver SC. 2008. Arbovirus evolution in vivo is constrained by host alternation. Proc Nat Acad Sci USA 105(19):6970–6975.

Coleman JR, Papamichail D, Skiena S, Futcher B, Wimmer E, Mueller S. 2008. Virus attenuation by genome-scale changes in codon pair bias. Science 320(5884):1784–1787.

Crameri G, Durr PA, Klein R, Foord A, Yu M, Riddell S, Haining J, Johnson D, Hemida MG, Barr J, Peiris M. 2016. Experimental infection and response to rechallenge of alpacas with Middle East respiratory syndrome coronavirus. Emerg Infect Dis 22(6):1071.

Cromer D, Grimm AJ, Schlub TE, Mak J, Davenport MP. 2016. Estimating the in-vivo HIV template switching and recombination rate. AIDS 30(2):185–192.

Cuevas JM, González-Candelas F, Moya A, Sanjuán R. 2009. Effect of ribavirin on the mutation rate and spectrum of hepatitis C virus in vivo. J Virol 83(11):5760–5764.

Cui P, Zhou D, Wu Y, Wu J, Lei J, Shao M, Liu G, Wu X, Wu J, Ji W. 2014. The ecological risks of avian influenza virus spread via migratory birds: a case study at Poyang Lake, China. Pak J Zool 46(6):1545–1555.

Davies TJ, Pedersen AB. 2008. Phylogeny and geography predict pathogen community similarity in wild primates and humans. Proc R Soc Lond B: Biol Sci 275(1643):1695–1701.

Diallo D, Sall AA, Buenemann M, Chen R, Faye O, Diagne CT, Faye O, Ba Y, Dia I, Watts D. 2012. Landscape ecology of sylvatic Chikungunya virus and mosquito vectors in southeastern Senegal. PLoS Negl Trop Dis 6(6):e1649.

Dimala CA, Atashili J, Mbuagbaw JC, Wilfred A, Monekosso GL. 2016. A comparison of the diabetes risk score in HIV/AIDS patients on highly active antiretroviral therapy (HAART) and HAART-naïve patients at the Limbe Regional Hospital, Cameroon. PLoS One 11(5):e0155560.

Diuk-Wasser MA, Brown HE, Andreadis TG, Fish D. 2006. Modeling the spatial distribution of mosquito vectors for West Nile virus in Connecticut, USA. Vector Borne Zoonotic Dis 6(3):283–295.

Domingo E, Perales C. 2016. Viral quasispecies and lethal mutagenesis. Eur Rev 24(01):39–48.

D'Ortenzio E, Matheron S, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, Descamps D, Damond F, Yazdanpanah Y, Leparc-Goffart I. 2016. Evidence of sexual transmission of Zika virus. N Engl J Med 374(22):2195–2198.

Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Gloza-Rausch F, Cottontail VM, Rasche A, Yordanov S. 2012. Bats host major mammalian paramyxoviruses. Nat Commun 3:796.

Duffy S, Shackelton L, Holmes EC. 2008. Rates of evolutionary changes in viruses: Patterns and determinants. Nat Rev Genet 9:267–276.

Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X, Scherbakova S, Graham RL, Baric RS, Stockwell TB. 2010. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. PLoS Pathog 6(5):e1000896.

Eckert KA, Kunkel TA. 1993. Fidelity of DNA synthesis catalyzed by human DNA polymerase α and HIV-1 reverse transcriptase: Effect of reaction pH. Nucleic Acids Res 21(22):5212–5220.

Eisen L, Eisen RJ. 2011. Using geographic information systems and decision support systems for the prediction, prevention, and control of vector-borne diseases. Annu Rev Entomol 56:41–61.

Fajardo A, Soñora M, Moreno P, Moratorio G, Cristina J. 2016. Bayesian coalescent inference reveals high evolutionary rates and diversification of Zika virus populations. J Med Virol 88:1672–1676.

Feldmann H. 2014. Ebola—a growing threat? N Engl J Med 371(15):1375–1378.

Fischer D, Thomas SM, Neteler M, Tjaden NB, Beierkuhnlein C. 2014. Climatic suitability of Aedes albopictus in Europe referring to climate change projections: Comparison of mechanistic and correlative niche modelling approaches. Euro Surveill 19(6):20696.

Fong IW. 2017. Emerging Animal Coronaviruses: First SARS and Now MERS. In: Emerging Zoonoses. Springer International Publishing. p. 63–80.

Fontana RJ. 2009. Side effects of long-term oral antiviral therapy for hepatitis. B Hepatology 49(S5):S185–S195.

Foster PL. 2006. Methods for determining spontaneous mutation rates. Methods Enzymol 409:195–213.

Gao D, Lou Y, He D, Porco TC, Kuang Y, Chowell G, Ruan S. 2016. Prevention and control of Zika as a mosquito-borne and sexually transmitted disease: A mathematical modeling analysis. Sci Rep 6:28070.

Geoghegan JL, Senior AM, Di Giallonardo F, Holmes EC. 2016. Virological factors that increase the transmissibility of emerging human viruses. Proc Nat Acad Sci USA 113(15):4170–4175.

Gilbert L, Walker L. 2010. 'My biggest fear was that people would reject me once they knew my status…': Stigma as experienced by patients in an HIV/AIDS clinic in Johannesburg, South Africa. Health Soc Care Community 18(2):139–146.

Gilleard JS. 2013. Haemonchus contortus as a paradigm and model to study anthelmintic drug resistance. Parasitology 140(12):1506–1522.

Gilsdorf A, Boxall N, Gasimov V, Agayev I, Mammadzade F, Ursu P, Gasimov E, Brown C, Mardel S, Jankovic D. 2005. Two clusters of human infection with influenza A/H5N1 virus in the Republic of Azerbaijan, February-March 2006. Euro Surveill 11(5):122–126.

Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, Jalloh S, Momoh M, Fullah M, Dudas G. 2014. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345(6202):1369–1372.

Gnädig NF, Beaucourt S, Campagnola G, Bordería AV, Sanz-Ramos M, Gong P, Blanc H, Peersen OB, Vignuzzi M. 2012. Coxsackievirus B3 mutator strains are attenuated in vivo. Proc Nat Acad Sci USA 109(34):E2294–E2303.

Greatorex ZF, Olson SH, Singhalath S, Silithammavong S, Khammavong K, Fine AE, Weisman W, Douangngeun B, Theppangna W, Keatts L, Gilbert M. 2016. Wildlife trade and human health in Lao PDR: An assessment of the zoonotic disease risk in markets. PLoS One 11(3):e0150666.

Hampton T. 2005. Bats may be SARS reservoir. JAMA 294(18): 2291–2291.

Haydon DT, Cleaveland S, Taylor LH, Laurenson M. 2002. Identifying reservoirs of infection: A conceptual and practical challenge. Emerg Infect Dis 8(12):1468–1473.

Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, Carroll MW, Doumbia M, Draguez B, Duraffour S, Enwere G. 2015. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: Interim results from the Guinea ring vaccination cluster-randomised trial. Lancet 386(9996):857–866.

Herlocher ML, Elias S, Truscon R, Harrison S, Mindell D, Simon C, Monto AS. 2001. Ferrets as a transmission model for influenza: Sequence changes in HA1 of type A (H3N2) virus. J Infect Dis 184(5):542–546.

Hoenen T, Safronetz D, Groseth A, Wollenberg K, Koita O, Diarra B, Fall I, Haidara F, Diallo F, Sanogo M. 2015. Mutation rate and genotype variation of Ebola virus from Mali case sequences. Science 348(6230):117–119.

Holmes EC. 2009. The evolutionary genetics of emerging viruses. Annu Rev Ecol Evol Syst 40:353–372.

Holmes EC. 2010. The RNA virus quasispecies: Fact or fiction? J Mol Biol 400(3):271–273.

Holmes EC. 2013. What can we predict about viral evolution and emergence? Curr Opin Virol 3(2):180–184.

Hotez PJ. 2017. Developing and financing neglected disease vaccines in our new era of "blue marble health" and the anthropocene epoch. Vaccine doi: 10.1016/j.vaccine.2017.02.002. [Epub ahead of print].

Hughes D, Andersson DI. 2015. Evolutionary consequences of drug resistance: Shared principles across diverse targets and organisms. Nat Rev Genet 16(8):459–471.

Hurt AC. 2014. The epidemiology and spread of drug resistant human influenza viruses. Curr Opin Virol 8:22–29.

Infectious Diseases Society of America. 2011. Combating antimicrobial resistance: Policy recommendations to save lives. Clin Infect Dis 52(suppl 5):S397–S428.

Jácome R, Becerra A, de León SP, Lazcano A. 2015. Structural analysis of monomeric RNA-dependent polymerases: Evolutionary and therapeutic implications. PLoS One 10(9): e0139001.

Jacque J-M, Triques K, Stevenson M. 2002. Modulation of HIV-1 replication by RNA interference. Nature 418(6896):435–438.

Johnson SF, Telesnitsky A. 2010. Retroviral RNA dimerization and packaging: The what, how, when, where, and why. PLoS Pathog 6(10):e1001007.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. 2008. Global trends in emerging infectious diseases. Nature 451(7181):990–993.

Joseph U, Su YC, Vijaykrishna D, Smith GJ. 2016. The ecology and adaptive evolution of influenza A interspecies transmission. Influenza Other Respir Viruses 11(1):74–84.

Katz IT, Ryu AE, Onuegbu AG, Psaros C, Weiser SD, Bangsberg DR, Tsai AC. 2013. Impact of HIV-related stigma on treatment adherence: Systematic review and meta-synthesis. J Int AIDS Soc 16(3):18640.

Kida H, Ito T, Yasuda J, Shimizu Y, Itakura C, Shortridge KF, Kawaoka Y, Webster RG. 1994. Potential for transmission of avian influenza viruses to pigs. J Gen Virol 75(9):2183–2188.

Klein EY, Serohijos AW, Choi J-M, Shakhnovich EI, Pekosz A. 2014. Influenza A H1N1 pandemic strain evolution–divergence and the potential for antigenic drift variants. PLoS One 9(4):e93632.

Kondrashov FA, Kondrashov AS. 2010. Measurements of spontaneous rates of mutations in the recent past and the near future. R Soc Lond B Biol Sci 365(1544):1169–1176.

Koval CE, Dykes C, Wang J, Demeter LM. 2006. Relative replication fitness of efavirenz-resistant mutants of HIV-1: Correlation with frequency during clinical therapy and evidence of compensation for the reduced fitness of K103N+ L100I by the nucleoside resistance mutation L74V. Virology 353(1):184–192.

Kožíšek M, Henke S, Šašková KG, Jacobs GB, Schuch A, Buchholz B, Müller V, Kräusslich H-G, Řezáčová P, Konvalinka J. 2012. Mutations in HIV-1 gag and pol compensate for the loss of viral fitness caused by a highly mutated protease. Antimicrob Agents Chemother 56(8):4320–4330.

Kunkel TA. 2004. DNA replication fidelity. J Biol Chem 279(17): 16895–16898.

Larder BA, Kellam P, Kemp SD. 1993. Convergent combination therapy can select viable multidrug-resistant HIV-1 in vitro. Nature 365(6445):451–453.

Lau SK, Li KS, Huang Y, Shek CT, Tse H, Wang M, Choi GK, Xu H, Lam CS, Guo R, Chan KH. 2010. Ecoepidemiology and complete genome comparison of different strains of severe acute respiratory syndrome-related Rhinolophus Bat Coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. J Virol 84:2808–2819.

Lauring AS, Andino R. 2010. Quasispecies theory and the behavior of RNA viruses. PLoS Pathog 6(7):e1001005.

Lee H, Popodi E, Tang H, Foster PL. 2012. Rate and molecular spectrum of spontaneous mutations in the bacterium Escherichia coli as determined by whole-genome sequencing. Proc Nat Acad Sci USA 109(41):E2774–E2783.

Lenhart A, Castillo C, Oviedo M, Villegas E. 2013. Use of the pupal/demographic-survey technique to identify the epidemiologically important types of containers producing Aedes aegypti (L.) in a dengue-endemic area of Venezuela. Ann Trop Med Parasitol 100(Suppl 1):S53–S59.

Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E. 2010. Bat guano virome: Predominance of dietary viruses from insects and plants plus novel mammalian viruses. J Virol 84(14):6955–6965.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426(6965):450–454.

Liu H, Gao XY, Fu SH, Li MH, Zhai YG, Meng WS, Sun XH, Lv Z, Wang HY, Shen XX, Cao YX. 2016. Molecular evolution of emerging Banna virus. Infect Genet Evol 45:250–255.

Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E. 2006 Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 12(12):1888–1894.

Lucas GM. 2005. Antiretroviral adherence, drug resistance, viral fitness and HIV disease progression: A tangled web is woven. J Antimicrob Chemother 55(4):413–416.

Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, Pulliam JR, Mills JN, Timonin ME, Willis CK, Cunningham AA, Fooks AR. 2013. A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? Proc R Soc B Biol Sci 280 (1756):20122753.

Mansky LM. 1996. Forward mutation rate of human immunodeficiency virus type 1 in a T lymphoid cell line. AIDS Res Hum Retr 12(4):307–314.

Maslow JN. 2017. Vaccine development for emerging virulent infectious diseases. Vaccine doi: 10.1016/j.vaccine.2017.02.015. [Epub ahead of print].

McGeoch DJ, Gatherer D. 2005. Integrating reptilian herpesviruses into the family Herpesviridae. J Virol 79:725–731.

Memoli MJ, Davis AS, Proudfoot K, Chertow DS, Hrabal RJ, Bristol T, Taubenberger JK. 2010. Multidrug-resistant 2009 pandemic influenza A (H1N1) viruses maintain fitness and transmissibility in ferrets. J Infect Dis 203(3):348–357.

Miguel E, Grosbois V, Caron A, Boulinier T, Fritz H, Cornélis D, Foggin C, Makaya PV, Tshabalala PT, de Garine-Wichatitsky M. 2013. Contacts and foot and mouth disease transmission from wild to domestic bovines in Africa. Ecosphere 4(4): 1–32.

Minskaia E, Hertzig T, Gorbalenya AE, Campanacci V, Cambillau C, Canard B, Ziebuhr J. 2006. Discovery of an RNA virus $3' \rightarrow 5'$ exoribonuclease that is critically involved in coronavirus RNA synthesis. Proc Nat Acad Sci USA 103 (13):5108–5113.

Mizokami M, Yokosuka O, Takehara T, Sakamoto N, Korenaga M, Mochizuki H, Nakane K, Enomoto H, Ikeda F, Yanase M. 2015. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naive and previously treated Japanese patients with genotype 1 Hepatitis C: An open-label, randomised, phase 3 trial. Lancet Infect Dis 15(6):645–653.

Mohd Jaafar F, Attoui H. 2015. Coltiviruses and Seadornaviruses. eLS. 1–12.

Morens DM, Folkers GK, Fauci AS. 2004. The challenge of emerging and re-emerging infectious diseases. Nature 430(6996): 242–249.

Morse SS, Mazet JA, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, Daszak P. 2012. Prediction and prevention of the next pandemic zoonosis. Lancet 380(9857):1956–1965.

Muñoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Pérez MJ, Sánchez-Somolinos M, Rincón C, Hortal J, Peláez T. 2005. Saccharomyces cerevisiae fungemia: An emerging infectious disease. Clin Infec Dis 40(11):1625–1634.

Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. 2015. Potential sexual transmission of Zika virus. Emerg Infect Dis 21(2):359–361.

Nakagawa F, May M, Phillips A. 2013. Life expectancy living with HIV: Recent estimates and future implications. Curr Opin Infect Dis 26(1):17–25.

Nguyen JT, Smee DF, Barnard DL, Julander JG, Gross M, de Jong MD, Went GT. 2012. Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. PLoS One 7(1):e31006.

Novella IS, Presloid JB, Taylor RT. 2014. RNA replication errors and the evolution of virus pathogenicity and virulence. Curr Opin Virol 9:143–147.

Obenauer JC, Denson J, Mehta PK, Su X, Mukatira S, Finkelstein DB, Xu X, Wang J, Ma J, Fan Y. 2006. Large-scale sequence analysis of avian influenza isolates. Science 311(5767): 1576–1580.

O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DT, Luis AD, Peel AJ, Plowright RK, Wood JL. 2014. Bat flight and zoonotic viruses. Emerg Infect Dis 20(5):741.

Ostfeld RS, Holt RD. 2004. Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. Front Ecol Environ 2(1):13–20.

Pérez-Losada M, Arenas M, Galán JC, Palero F, González-Candelas F. 2015. Recombination in viruses: Mechanisms, methods of study, and evolutionary consequences. Infect Genet Evol 30:296–307.

Pérez-Torrado R, Querol A. 2015. Opportunistic strains of Saccharomyces cerevisiae: A potential risk sold in food products. Front Microbiol 6:1522.

Peterson AT, Vieglais DA, Andreasen JK. 2003. Migratory birds modeled as critical transport agents for West Nile virus in North America. Vector Borne Zoonotic Dis 3(1):27–37.

Pfeiffer JK, Kirkegaard K. 2003. A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity. Proc Nat Acad Sci USA 100(12):7289–7294.

Powell JR, Tabachnick WJ. 2013. History of domestication and spread of Aedes aegypti-A Review. Mem Inst Oswaldo Cruz 108:11–17.

Price MN, Arkin AP. 2015. Weakly deleterious mutations and low rates of recombination limit the impact of natural selection on bacterial genomes. MBio 6(6):e01302–e01315.

Price SJ, Garner TW, Cunningham AA, Langton TE, Nichols RA. 2016. Reconstructing the emergence of a lethal infectious disease of wildlife supports a key role for spread through translocations by humans. Proc Biol Sci 283(1839):20160952.

Quattrochi D, Hayden M, Crosson WL, Lozano-Fuentes S, Eisen L, Irwin DE, Estes MG Jr, Moreno-Madriñán MJ, Monaghan AJ, Welsh-Rodriguez CM. 2014. Correlating remote sensing data with the abundance of pupae of the Dengue Virus mosquito vector, Aedes aegypti, in Central Mexico.

Reed C, Bruden D, Byrd KK, Veguilla V, Bruce M, Hurlburt D, Wang D, Holiday C, Hancock K, Ortiz JR. 2014. Characterizing wild bird contact and seropositivity to highly pathogenic avian influenza A (H5N1) virus in Alaskan residents. Influenza Other Respir Viruses 8(5):516–523.

Reiter P, Sprenger D. 1987. The used tire trade: A mechanism for the worldwide dispersal of container breeding mosquitoes. J Am Mosq Control Assoc 3(3):494–501.

Reynolds JJ, Torremorell M, Craft ME. 2014. Mathematical modeling of influenza A virus dynamics within swine farms and the effects of vaccination. PLoS One 9(8):106177.

Richard L, Mouinga-Ondémé A, Betsem E, Filippone C, Nerrienet E, Kazanji M, Gessain A. 2016. Zoonotic transmission of two new strains of human T-lymphotropic virus type 4 in hunters bitten by a gorilla in Central Africa. Clin Infect Dis 63(6):800–803.

Rodríguez-Morales AJ, Paniz-Mondolfi AE, Villamil-Gómez WE, Navarro JC. 2016. Mayaro, Oropouche and Venezuelan Equine Encephalitis viruses: Following in the footsteps of Zika? Travel Med Infect Dis 15:72–73.

Rosenberg R. 2015. Detecting the emergence of novel, zoonotic viruses pathogenic to humans. Cell Mol Life Sci 72(6): 1115–1125.

Røttingen JA, Gouglas D, Feinberg M, Plotkin S, Raghavan KV, Witty A, Draghia-Akli R, Stoffels P, Piot P. 2017. New vaccines against epidemic infectious diseases. N Engl J Med 376 (7):610–613.

Rozema DB, Lewis DL, Wakefield DH, Wong SC, Klein JJ, Roesch PL, Bertin SL, Reppen TW, Chu Q, Blokhin AV. 2007. Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. Proc Nat Acad Sci USA 104(32):12982–12987.

Rozen-Gagnon K, Stapleford KA, Mongelli V, Blanc H, Failloux A-B, Saleh M-C, Vignuzzi M. 2014. Alphavirus mutator variants present host-specific defects and attenuation in mammalian and insect models. PLoS Pathog 10(1):e1003877.

Saéz AM, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Düx A, Kühl HS, Kaba M, Regnaut S, Merkel K, Sachse A. 2015. Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med 7(1):17–23.

Sanjuán R, Domingo-Calap P. 2016. Mechanisms of viral mutation. Cell Mol Life Sci 73(23):4433–4448.

Schlub TE, Grimm AJ, Smyth RP, Cromer D, Chopra A, Mallal S, Venturi V, Waugh C, Mak J, Davenport MP. 2014. Fifteen to twenty percent of HIV substitution mutations are associated with recombination. J Virol 88(7):3837–3849.

Schrag SJ, Rota PA, Bellini WJ. 1999. Spontaneous mutation rate of measles virus: Direct estimation based on mutations conferring monoclonal antibody resistance. J Virol 73(1):51–54.

Seo S, Englund JA, Nguyen JT, Pukrittayakamee S, Lindegardh N, Tarning J, Tambyah PA, Renaud C, Went GT, De Jong MD. 2013. Combination therapy with amantadine, oseltamivir and ribavirin for influenza A infection: Safety and pharmacokinetics. Antivir Ther 18(3):377.

Sharp PM, Hahn BH. 2011. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med 1(1):a006841.

Shriner D, Rodrigo AG, Nickle DC, Mullins JI. 2004. Pervasive genomic recombination of HIV-1 in vivo. Genetics 167(4): 1573–1583.

Simon-Loriere E, Holmes EC. 2011. Why do RNA viruses recombine? Nat Rev Microbiol 9(8):617–626.

Smith EC, Blanc H, Vignuzzi M, Denison MR. 2013. Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: Evidence for proofreading and potential therapeutics. PLoS Pathog 9(8):e1003565.

Song D, Kang B, Lee C, Jung K, Ha G, Kang D, Park S, Park B, Oh J. 2008. Transmission of avian influenza virus (H3N2) to dogs. Emerg Infect Dis 14:741–746.

Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards JAmerica IDSo. 2008. The epidemic of antibiotic-resistant infections: A call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 46(2):155–164.

Stahl JP, Mailles A, Dacheux L, Morand P. 2011. Epidemiology of viral encephalitis in 2011. Méd Mal Infect 41(9):453–464.

Steel N. 2016. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: The Global Burden of Disease Study 2015. Lancet HIV 3(8): e361–e387.

Streicker DG, Turmelle AS, Vonhof MJ, Kuzmin IV, McCracken GF, Rupprecht CE.2010. Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. Science 329(5992):676–679.

Stringer KL, Turan B, McCormick L, Durojaiye M, Nyblade L, Kempf M-C, Lichtenstein B, Turan JM. 2016. HIV-related stigma among healthcare providers in the deep south. AIDS Behav 20(1):115–125.

Tamura D, DeBiasi RL, Okomo-Adhiambo M, Mishin VP, Campbell AP, Loechelt B, Wiedermann BL, Fry AM, Gubareva LV. 2015. Emergence of multidrug-resistant influenza A (H1N1) pdm09 virus variants in an immunocompromised child treated with Oseltamivir and Zanamivir. J Infect Dis 212(8):1209–1213.

Taubenberger JK, Morens DM. 2010. Influenza: The once and future pandemic. Public Health Rep 125(Suppl 3):16–26.

Tompkins SM, Lo C-Y, Tumpey TM, Epstein SL. 2004. Protection against lethal influenza virus challenge by RNA interference in vivo. Proc Nat Acad Sci USA 101(23):8682–8686.

Tong S, Li Y, Rivailler P, Conrardy C, Castillo DAA, Chen L-M, Recuenco S, Ellison JA, Davis CT, York IA. 2012. A distinct lineage of influenza A virus from bats. Proc Nat Acad Sci USA 109(11):4269–4274.

Tsetsarkin KA, Chen R, Weaver SC. 2016. Interspecies transmission and Chikungunya virus emergence. Curr Opin Virol 16: 143–150.

Varble A, Albrecht RA, Backes S, Crumiller M, Bouvier NM, Sachs D, García-Sastre A. 2014. Influenza A virus transmission bottlenecks are defined by infection route and recipient host. Cell Host Microbe 16(5):691–700.

Vignuzzi M, Stone JK, Arnold JJ, Cameron CE, Andino R. 2006. Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. Nature 439 (7074):344–348.

Villinger J, Mbaya MK, Ouso D, Kipanga PN, Lutomiah J, Masiga DK. 2016. Arbovirus and insect-specific virus discovery in Kenya by novel six genera multiplex high-resolution melting analysis. Mol Ecol Resour 17(3):466–480.

Vogler AJ, Chan F, Nottingham R, Andersen G, Drees K, Beckstrom-Sternberg SM, Wagner DM, Chanteau S, Keim P. 2013. A decade of plague in Mahajanga, Madagascar: Insights into the global maritime spread of pandemic plague. MBio 4(1):e00623–12.

Vuilleumier S, Bonhoeffer S. 2015. Contribution of recombination to the evolutionary history of HIV. Curr Opin HIV AIDS 10(2):84–89.

Warfield KL, Dye JM, Wells JB, Unfer RC, Holtsberg FW, Shulenin S, Vu H, Swenson DL, Bavari S, Aman MJ. 2015. Homologous and heterologous protection of nonhuman primates by Ebola and Sudan virus-like particles. PLoS One 10(3):e0118881.

Watanabe T, Zhong G, Russell CA, Nakajima N, Hatta M, Hanson A, McBride R, Burke DF, Takahashi K, Fukuyama S. 2014. Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. Cell Host Microbe 15(6):692–705.

Weaver SC. 2013. Urbanization and geographic expansion of zoonotic arboviral diseases: Mechanisms and potential strategies for prevention. Trends Microbiol 21(8):360–363.

Weaver SC, Forrester NL. 2015. Chikungunya: Evolutionary history and recent epidemic spread. Antiviral Res 120: 32–39.

Weaver SC, Osorio JE, Livengood JA, Chen R, Stinchcomb DT. 2012. Chikungunya virus and prospects for a vaccine. Expert Rev Vaccines 11(9):1087–1101.

Weber DJ, Rutala WA, Fischer WA, Kanamori H, Sickbert-Bennett EE. 2016. Emerging infectious diseases: Focus on infection control issues for novel coronaviruses (Severe Acute Respiratory Syndrome-CoV and Middle East Respiratory Syndrome-CoV), hemorrhagic fever viruses (Lassa and Ebola), and highly pathogenic avian influenza viruses, A (H5N1) and A (H7N9). Am J Infect Control 44(5): e91–e100.

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. 1992. Evolution and ecology of influenza A viruses. Microbiol Rev 56(1):152–179.

Webster RG, Monto AS, Braciale TJ, Lamb RA. 2014. Textbook of influenza. John Wiley & Sons.

Weverling GJ, Lange JM, Jurriaans S, Prins JM, Lukashov VV, Notermans DW, Roos M, Schuitemaker H, Hoetelmans RM,

Danner SA. 1998. Alternative multidrug regimen provides improved suppression of HIV-1 replication over triple therapy. Aids 12(11):F117–F122.

Wong SK, Li W, Moore MJ, Choe H, Farzan M. 2004. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 279(5):3197–3201.

Woolhouse ME, Brierley L, McCaffery C, Lycett S. 2016. Assessing the epidemic potential of RNA and DNA viruses. Emerg Infect Dis 22(12):2037.

Woolhouse ME, Gowtage-Sequeria S. 2005. Host range and emerging and reemerging pathogens. Emerg Infect Dis 11(12):1842.

Wu JT, Cowling BJ. 2011. The use of mathematical models to inform influenza pandemic preparedness and response. Exp Biol Med 236(8):955–961.

Yim HJ, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS. 2006. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology 44(3):703–712.

Zehender G, Frati ER, Martinelli M, Bianchi S, Amendola A, Ebranati E, Ciccozzi M, Galli M, Lai A, Tanzi E. 2016. Dating the origin and dispersal of Human Papillomavirus type 16 on the basis of ancestral human migrations. Infect Gen Evol 39:258–264.

Zeller HG, Schuffenecker I. 2004. West Nile virus: An overview of its spread in Europe and the Mediterranean basin in contrast to its spread in the Americas. Eur J Clin Microbiol Infect Dis 23(3):147–156.

Zhang Z, Shen L, Gu X. 2016. Evolutionary dynamics of MERS-CoV: Potential recombination, positive selection and transmission. Sci Rep 6:25409.

Zhu H, Wang D, Kelvin D, Li L, Zheng Z, Yoon S-W, Wong S-S, Farooqui A, Wang J, Banner D. 2013. Infectivity, transmission, and pathology of human-isolated H7N9 influenza virus in ferrets and pigs. Science 341(6142):183–186.

Zirkel F, Kurth A, Quan P-L, Briese T, Ellerbrok H, Pauli G, Leendertz FH, Lipkin WI, Ziebuhr J, Drosten C. 2011. An insect nidovirus emerging from a primary tropical rainforest. MBio 2(3):e00077–11.