MINI-REVIEW

Animal and human RNA viruses: genetic variability and ability to overcome vaccines

T. G. Villa[1] · Ana G. Abril[1] · S. Sánchez[1] · T. de Miguel[1] · A. Sánchez-Pérez[2]

Abstract

RNA viruses, in general, exhibit high mutation rates; this is mainly due to the low fidelity displayed by the RNA-dependent polymerases required for their replication that lack the proofreading machinery to correct misincorporated nucleotides and produce high mutation rates. This lack of replication fidelity, together with the fact that RNA viruses can undergo spontaneous mutations, results in genetic variants displaying different viral morphogenesis, as well as variation on their surface glyco-proteins that affect viral antigenicity. This diverse viral population, routinely containing a variety of mutants, is known as a viral 'quasispecies'. The mutability of their virions allows for fast evolution of RNA viruses that develop antiviral resistance and overcome vaccines much more rapidly than DNA viruses. This also translates into the fact that pathogenic RNA viruses, that cause many diseases and deaths in humans, represent the major viral group involved in zoonotic disease transmission, and are responsible for worldwide pandemics.

Keywords RNA viruses · Viral RNA polymerases · Viral genetic variability · Viral vaccines

Introduction

Animal RNA viruses, in general, exhibit high mutation rates; according to Combe and Sanjuán (2014), the frequency of new mutations in these virions ranges from 10^{-4} to 10^{-6} substitutions per nucleotide per round of copying, with transitions more common than transversions. In addition, the mutation process is also affected by the host cells, with VSV mutating at a significantly higher rate in mammalian cells than in insect cells. Other factors affecting the frequency of mutation are the viral family, the polarity of the RNA ($+$ or positive-sense, 5′-to-3′;—or negative-sense, 3′-to-5′), and whether the genome is single-stranded (ssRNA) or double-stranded (dsRNA). The RNA-dependent RNA polymerases in these viruses lack the proofreading machinery to correct misincorporated nucleotides, which often results in a high generation of mutated phenotypes. This means that RNA viruses exhibit good abilities to overcome the pressure of either vaccines or antiviral drugs; these viruses have an advantage over DNA viruses in this regard, as the latter contain proofreading mechanisms. Even if infection is initiated by a single pathogenic RNA-containing unit, after a few rounds of replication, the viral population routinely contains a variety of mutants (known as "the mutant swarm"; Novella et al. 1995); at least in theory, the mutants originated will vary in their sensitivity to both the antibodies produced by the host and the antivirals used in therapy. All these variants are known as a viral 'quasispecies' (Holland et al. 1992), they contain a substantial variety of genomes, and constitute the reason why RNA viruses evolve from thousands to millions of times faster than DNA-based organisms. This is of particular relevance for vaccines and antivirals, making it imperative to extend the research into plant and animal viruses to develop new antiviral drugs, to keep abreast of the viruses developing resistance, as well as appropriately updating the relevant vaccines (Domingo et al. 1997). Fortunately, both plants and animals exhibit an old antiviral system (post-transcriptional gene silencing) that, to a certain extent, can withstand the mutation proclivity of RNA viruses (Marathe et al. 2000).

Communicated by Erko stackebrandt.

✉ T. G. Villa
tomas.gonzalez@usc.es

[1] Department of Microbiology, Faculty of Pharmacy, University of Santiago de Compostela, 5706 Santiago de Compostela, Spain

[2] Sydney School of Veterinary Science, Faculty of Science, University of Sydney, Sydney, NSW 2006, Australia

Isaacs and Lindenmann reported in 1957 a substance with antiviral activity, synthesized by embryonic hen eggs infected with the influenza virus, that they denominated "interferon" (IFN). This pioneering work opened the door to the study of antiviral compounds produced by higher cells and led to the current classification of interferons as signaling proteins (cytokines) capable of selectively activating the immune system; these defensive mechanisms include the activation of natural killer cells and macrophages, as well as an increased expression of the major histocompatibility complex antigens. Interferons are organized into three groups, Type I, Type II and Type III. Type I INFs, previously known as acid-resistant IFNs, are glycosylated or phosphorylated proteins that include nine subtypes, IFN-α, IFN-β, IFN-δ, IFN-ε, IFN-ζ, IFN-κ, IFN-ν, IFN-τ, and IFN-ω (Dussurget et al. 2014), and are produced by leukocytes exposed to pathogens. Type II IFN, also known as immune interferon or γ interferon, is an acid-labile lipoprotein that spans 166 amino acids in humans; while the less studied Type III IFN was originally described as an antiviral protein by Wheelock and Sibley (1965). Since interferon constitutes one of the main barriers against viral infections in vertebrates, it is essential to identify viral mutations that render viruses resistant to IFNs.

Figure 1 depicts the different viral families included in this article, while Table 1 summarizes the characteristics of the viruses.

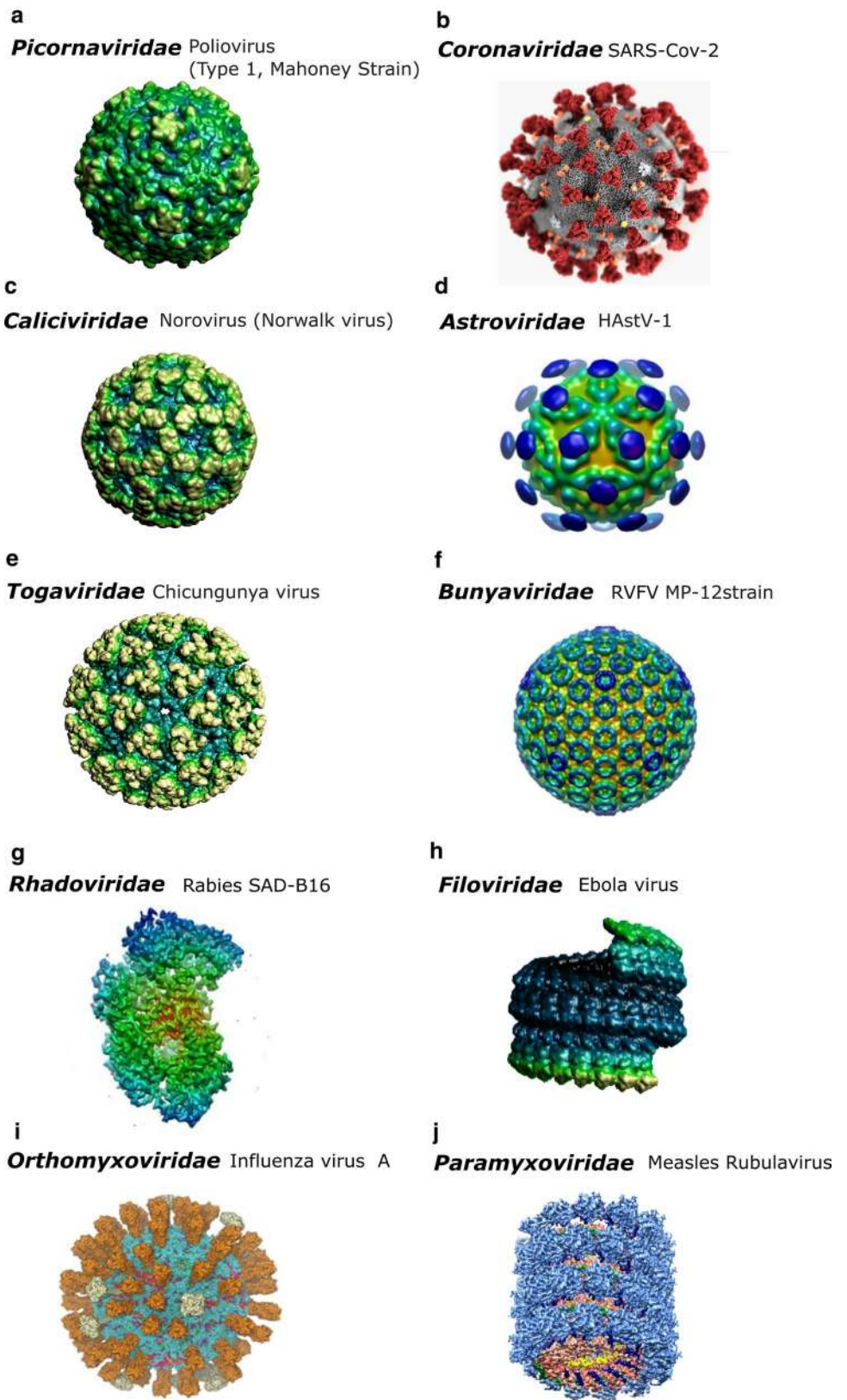## Positive-sense single-stranded RNA viruses (+ssRNA)

The positive-sense single-stranded RNA present in these viruses works as an mRNA when it reaches the cytoplasm of the cells it infects. Eukaryotic mRNAs have a methylated cap structure that is essential for their stability and protein translation (Furuichi and Shatkin 2000). During virus-host co-evolution, viruses developed different strategies to modify their RNAs to mimic eukaryotic mRNAs, to escape detection by the innate immune system and to ensure efficient protein translation. The two main approaches involve either covalently attach a peptide or a 7-methylguanosine cap to the first nucleotide at the 5'-end of their RNAs. Viral families, such as *Picornaviridae*, *Caliciviridae* and *Astroviridae*, covalently attach a protein known as VPg (viral protein genome-linked), while *Coronaviridae* and *Togaviridae* attach a cap, via a triphosphate bridge (Reviewed by Decroly et al. 2012). Coronaviruses encode their own capping enzymes and follow the canonical capping pathway, consisting of four sequential enzymatic reactions, resulting on a m7GpppAm-cap (Sun et al. 2014; Sola et al. 2015). *Togaviridae* can also synthesize a viral cap identical to a eukaryotic cap, but follows an unconventional synthesis pathway (Decroly et al. 2012).

Picornaviruses

These are small positive-sense single-stranded RNA viruses (22–32 nm in diameter) that directly replicate in the cytoplasm of infected cells. The picornavirus + ssRNA is 7.2–9.0 kb long and contains a virally encoded protein (VPg) at the 5′ end that plays the role of a primer in transcription; it also possesses a poly (A) tail at its 3′end. Positive-sense RNA is similar to mRNA (displaying the same sense), which means that the nucleic acid can be directly translated by the host cell; this quality makes the naked + ssRNA putatively infectious, although to a lesser degree than the complete virion. RNA translation results in a single polyprotein, that is later cleaved to produce all the active viral proteins (Villa-Kumaroff et al. 1975). The icosahedral capsid of the virion contains 60 copies of each of four capsid proteins, VP1, VP2, VP3, and VP4; the first three are large proteins, whereas the smaller VP4 is located on the inner surface of the capsid, in contact with both the RNA and the amino termini of the polypeptides VP1 and VP3 (Mosser et al. 1994). Picornaviridae is a large family of viruses that includes the classic genera *Enterovirus* (encompassing the poliomyelitis causative agent), *Rhinovirus, Hepatovirus, Cardiovirus,* and *Aphtovirus*; in addition, there are a number of genera that were recently discovered, these incorporate *Kobuvirus* (Sasaki et al. 2001)*, Tremovirus* (Johansson et al. 2002)*, Teschovirus* (King et al. 2000)*, Parechovirus* (Joki-Korpela and Hyypiä 2001), and *Erbovirus* (Pringle 1999). The picornaviruses produce a variety of human diseases but, to date, there are only effective vaccines for poliomyelitis and hepatitis A. Vaccine production requires inactivated or attenuated viral strains and certain mutations in the viral genome can produce attenuated strains, hence, it is essential to identify these RNA mutations, as they could represent putative vaccines for pathogenic picornaviruses still lacking them.

Notable examples of picornavirus Polioviruses contain 3 antigenically distinct serotypes, type 1, 2 and 3, and all three are required for a vaccine to protect humans against the disease; correspondingly, study of their mutation rates as well as their genetic drift is essential to design effective vaccines. The article published by Richard I. Carp in 1963 represents (Carp, 1963), to the best of our knowledge, one of the earliest studies on the variation of the mutational rate in picornavirus. Carp studied two particular characteristics in several poliovirus strains, the reproductive temperature/40$^+$ (rct40$^+$) and guanidine dependent (g$^+$) characters, and concluded that the mutation rates varied between the different poliovirus strains studied. In addition, the variations in mutation rates encountered in different strains did not correlate with the number of steps required to attain the mutated character and, more significantly, did not correspond to the stability of the strain during passage in the human intestine.

Fig. 1 Graphic representation of the 10 human viruses described in the text (creative common licence figures from VIPERdb https://viperdb.scripps.edu (Carrillo-Tripp et al. 2009) and Reddy et al. 2015). The virus families included here are: **a** Picornaviridae, **b** Coronaviridae, **c** Caliciviridae, **d** Astroviridae, **e** Togaviridae, **f** Bunyaviridae, **g** Rhadoviridae, **h** Filoviridae, **i** Orthomyxoviridae, and **j** Paramyxoviridae

a
Picornaviridae Poliovirus (Type 1, Mahoney Strain)

b
Coronaviridae SARS-Cov-2

c
Caliciviridae Norovirus (Norwalk virus)

d
Astroviridae HAstV-1

e
Togaviridae Chicungunya virus

f
Bunyaviridae RVFV MP-12strain

g
Rhadoviridae Rabies SAD-B16

h
Filoviridae Ebola virus

i
Orthomyxoviridae Influenza virus A

j
Paramyxoviridae Measles Rubulavirus

Table 1 Characteristics of the main ssRNA human virus families (Dietzgen et al. 2017; Kuhn et al. 2019; Rima et al. 2019; Chen et al. 2018; Vinjé et al. 2019; Zell et al. 2017; Mandary et al. 2019)

Family	Picornaviridae	Coronaviridae	Caliciviridae	Astroviridae	Togaviridae	Bunyaviridae	Rhabdoviridae	Filoviridae	Orthomyxoviridae	Paramyxoviridae
Virion										
Diameter	22–32 nm	60–220 nm	27–40 nm	30 nm	65–70 nm	80–120 nm	75–180 nm	800–1000 nm in leght-80 nm diameter	50–120 nm	300–1000 nm
Envelope	Ne	E	Ne	Ne	E	E	E (not all)	E	E	E
Shape	s	s-pl (baciliform or tubular)	s	s	s	s-pl	u	pl	s-pl- f	s-p
Capsid symmetry	Icosahedral	Helical	Icosahedral	icosahedral	Icosahedral or cubic	Helical	Helical	Helical	Helical	Helical
Structural proteins	VP1, VP2, VP3, and VP4	Multiple copies of N and M	Major capsid protein, VP1, a minor structural protein, VP2 and VPg	VP26, VP29 y VP32	CP, E2, E1 and 3 GP	NC and two GP	N, P, M, G and L	NP, GP, and two matrix proteins, VP24 and VP40	NP, a hemagglutinin and a non-glycosylated matrix protein (M1 or M)	F protein, M protein and HN/G/H glycoprotein spikes and N
Genome										
Nº Capsomeres	32	–	32	32	80 trimers	–	–	–	–	–
Genome length	7.2 to 9.0 kb	20–30 kb	7.4–8.3 kb	7.0 kb	10–12 kb	11–22 kb	10–16 kb	15–19 kb	12-15 KB	14.6–20.1 kb
Segmentation	n-seg	n-seg	n-seg	n-seg	n-seg	seg	unsegmented or bi-segmented	n-seg	seg	n-seg
Polarity	p-sense	p-sense	p-sense	p-sense	p-sense	n-sense	n-sense	n-sense	n-sense	n-sense
Replication	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic, in vesicles derived from the plasma membrane/endosomal compartment	Cytoplasmic	Cytoplasmic	Cytoplasmic	Nucleus	Cytoplasmic

Table 1 (continued)

Family	Picornaviridae	Coronaviridae	Caliciviridae	Astroviridae	Togaviridae	Bunyaviridae	Rhabdoviridae	Filoviridae	Orthomyxoviridae	Paramyxoviridae
Host range	Vertebrates	Mammals, birds and fish	Mammals, birds, fish	Mammals and birds	Humans and non-human primates, equids, birds, amphibians, reptiles, rodents, pigs, sea mammals, salmonids, mosquitoes and some other arthropods; most alphaviruses are mosquito vector borne	Vertebrates, invertebrates and plants	Vertebrates, arthropods and plants	Primates, bats, domestic pigs, and probably fish become naturally infected	Humans, horses, pigs, mink, seals, whales, fowl and fish	Mammals, birds, fish and reptiles
Entry route and receptor	Endocytosis, cell surface, poliovirus receptor	Endocytosis, cell surface	Endocytosis, H type 2 histo-blood group antigen	Endocytosis	Endocytosis	Endocytosis, β3 integrins	Endocytosis, acetylcholine, receptor, neural cell, adhesion molecule	Endocytosis, Folate receptor-α	Endocytosis, sialic acid	Cell surface, sialic acid
Triangulation number	$p = 3$*	–	$T = 1$ or 3	$T = 3$	$T = 4$	–	–	–	–	–
Disease or syndrome	A variety of human diseases ranging from severe (poliomyelitis, encephalitis, meningitis, and hepatitis) to mild (common cold)	SARS, Respiratory-track infections and gastroenteritis	acute gastroenteritis (AGE), diarrhea	Acute gastroenteritis	Febrile illness, encephalitic and arthritic	Hemorrhagic fever, respiratory distress, pneumonia, encephalitis	Severe hemorrhagic disease and fatal encephalitis (rabies)	Severe hemorrhagic disease	Rhinitis, Bronchitis, Pneumonia, croup	Rhinitis, pneumonia, pharyngitis
Typical member	Poliovirus and Rhinovirus	SARS-CoV-2	Norwalk virus (M87661)	HAstV-1, HAstV-MLB2	Chikungunya virus and Rubella virus	RVFV MP-12 strain	Rabies virus	Marburg and Ebola virus	Influenza virus	Measles virus

Ne non enveloped, *E* enveloped, *s* spherical, *u* elongated with parallel sides and a round end, *pl* pleomorphic, *s-pl* spherical-pleomorphic, *s-pl-f* spherical-pleomorphic and filamentous form, *ps* positive-sense, *ns* negative-sense, *CP* capsid protein, *NP* nucleoprotein, *M* matrix or membrane protein, *GP* Glycoproteins, *n-seg* non-segmented RNA, *seg* segmented RNA, *n-sense* negative-sense, *p-sense* positive-sense, *IRES* internal ribosomal entry site, *HAstV-1* Human astrovirus serotype 1, *RVFV* Rift Valley fever virus

*The subunits are not exactly equivalent, pseudo-triangulation numbers referred as p

The poliovirus type 1 genome was completely sequenced by Kitamura and co-workers in 1981, demonstrating that the positive-sense RNA encodes a polyprotein of 247 kDa. Until 1982, little was known about the effect of mutations on the life cycle of polioviruses and, at that point, the only mutations characterized corresponded to the viral coat protein and produced defective-interfering particles. In 1982, Hewlett and colleagues were able to isolate six temperature sensitive mutants of poliovirus type 1, containing high number of hydroxylamine-generated mutations; these virions, despite the deleterious effects caused by the mutations, could still inhibit protein synthesis in the host, and this inhibition was just slightly lower than that produced by the wild-type poliovirus. However, Parvin and coworkers discovered in 1986 that the poliovirus genome is quite stable, at least if compared to that of the influenza virus; the authors reported that the mutation rate for the VP1 gene is less than 2.1×10^{-6} mutations per nucleotide per infectious cycle. Despite this relatively low mutation rate, Racaniello and Meriam described in 1986 the existence of point mutations that can substantially alter the viral production cycle; these authors demonstrated that, a single mutation in the 5-noncoding region, resulted in at least a 100-fold reduction in viral progeny, as compared to the wild-type virus. The strategy of artificially generating mutations at the 5′terminus was also successfully exploited by Izuka and co-workers in 1989, originating stable polioviruses that are less neurovirulent.

Dunn et al. (1990) studied poliovirus excretion by infants undergoing their first vaccination with live oral polio vaccine, and noted that the mutations in the 5′-noncoding region mentioned above, typical of attenuated phenotypes, reverted rapidly for type 3, and at a slower rate for type 1 viruses. Apart from induced mutations, polioviruses also exhibit spontaneous mutations that, according to Drake in (1993), are actually 300-fold higher than those previously reported for viral particles containing DNA genomes. Evidently, this fact must be taken into account when selecting poliovirus strains to use in vaccine preparation. In addition, Chumakov and colleagues, working with a Sabin-derived poliovirus type 3 strain, produced an interesting publication in 1994 demonstrating that virus revertants, originating from virions containing a point mutation at nucleotide 472, appeared at different rates depending on the strains used; astonishingly, the results suggested that host factors may be involved in the selection of the revertants.

The replication of RNA viruses usually depends on an RNA polymerase that copies the nucleic acid with low fidelity, resulting in a high frequency (ranging from 10^{-3} to 10^{-4}) of misincorporated nucleotides per round of replication. Polioviruses appear to have particularly high mutation rates, up to a million times higher than the organisms they infect. Mutations play an important role in viral adaptation

but, if too high, they are deleterious, hence the mutation rates of these enzymes could be expected to be optimized by natural selection. This appears not to be the case for polioviruses, described to have a selection for faster replicating enzymes, hence responsible for increased error rates (Duffy 2018). The poliovirus polymerase (3Dpol) is one of the best studied viral enzymes, it spans a GDD motif, as well as two regions denominated A and B. The GDD motif, also known as the C motif, contains two aspartic acid residues and is well conserved in viral RNA-dependent RNA polymerases; the A and B motifs are also well conserved. Of the two aspartic residues in GDD, the first one is essential for enzymatic activity; while substitution of the second amino acid by asparagine produces an enzyme that displays some activity under certain conditions (Jablonski and Morrow 1995). This fact could be relevant when analyzing reversions in poliovirus strains used for vaccine production, as is the case for Sabin 2 isolates (Taffs et al. 1995). Several studies have focused on the creation of variants for the "D motif", as it was suggested as a relevant target to obtain a variety of viral strains that could represent good vaccine candidates (Liu et al. 2013).

The error-prone nature of the RNA-dependent RNA polymerase could be also responsible for the generation of viral strains resistant to antivirals. Accordingly, Meng and Kwan reported in (2014) that the enterovirus 71, containing a L123F mutation in its RNA-dependent RNA polymerase, is an attenuated antiviral-resistant strain that could be used as an effective vaccine against this virus; this adds another layer on the subject of creating novel vaccines by utilizing and controlling the mutational strategy of the virus. In some picornavirus, such as foot and mouth disease virus (FMDV), Zeng and colleagues (2014) reported that higher replication fidelity is associated with attenuated viral strains; these candidates could represent good vaccine alternatives to the, currently used, chemically-inactivated strains.

Gitlin et al. (2002) identified short interfering RNAs (siRNA) as a new type of antivirals against polioviruses; these siRNA confer intracellular antiviral immunity in human cells, thus creating a new strategy to control poliovirus. Obviously, mutations in viral RNA can render these siRNAs obsolete, hence, no longer capable of controlling viral replication. In accordance, Schott et al. reported in 2005 that multiple RNA interference (RNAi) are required for cells to resist infection by vesicular stomatitis virus (VSV), suggesting that the cellular RNAi machinery plays a role in the fight against viruses in nature.

Coronaviridae

The *Coronaviridae* family (subfamily *Coronavirinae*) is included in the *Nidovirales* order (de Groot et al. 2011) and encompasses the genera *Alphacoronavius*, *Betacoronavirus,*

Gammacoronavirus, and *Deltacoronavirus*. These viruses contain positive-sense single-stranded RNA, with a virally encoded protein at the 5′end and a poly (A) tail at its 3′end. This is another viral family in which the positive-stranded nucleic acid works as an mRNA when it reaches the host cytoplasm, and the first protein translated is the RNA-dependent RNA polymerase. This replicase is assisted by a non-structural protein that has proofreading activity; this enzyme is different from those present in other positive-stranded RNA viruses, as it carries a mutation that enables it to resist a variety of mutagenic agents (Sexton et al. 2016; early work by Xu et al. 2003). As a result, both positive- and negative-stranded RNA populations generated by the virus in the successive replicative cycles are quite homogeneous; this implies that the genetic drift of these viruses is low, which facilitates vaccine design.

Coronavirus exhibit helical symmetry and contain the largest genome among RNA viruses. This group was discovered in the 1960s and named 'corona' (Latin name meaning crown) because of its microscopic appearance. The *Coronaviridae* display an S peplomer on the viral surface, this is a glycoprotein spike on the viral envelope that recognizes and reacts with the host receptor; the receptor contains N-acetylneuraminic acid and plays a major role in viral tropism. The virus replicates in the cytoplasm of the human respiratory tract cells, causing symptoms similar to those of the common cold. A variety of coronaviruses have been described, they are classified into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. The *Alphacoronavirus* include HCoV-229E, HCoV-NL63, *Miniopterus* bat coronavirus 1, *Miniopterus* bat coronavirus HKU8, porcine epidemic diarrhea virus, *Rhinolophus* bat coronavirus HKU2, and *Scotophilus* bat coronavirus 512. Some examples of *Betacoronavirus* are betacoronavirus 1, human coronavirus HKU1, murine coronavirus, *Pipistrellus* bat coronavirus HKU5, *Rousettus* bat coronavirus HKU9, severe acute respiratory syndrome coronavirus (SARS-CoV), *Tylonycteris* bat coronavirus HKU4, Middle East respiratory syndrome coronavirus (MERS-CoV), human coronavirus OC43 (HCoV-OC43), and hedgehog coronavirus 1 (EriCoV). Beluga whale coronavirus SW1 and infectious bronchitis virus IBV belong to the genus *Gammacoronavirus*, while the *Deltacoronavirus* genus includes HKU11 and HKU15.

The representative human coronaviruses include HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HKU1, and MERS-CoV. The latest identified human coronavirus is SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), also known as 2019-nCoV, "novel coronavirus 2019" and COVID-19 (Liu et al. 2017; Corman et al. 2018). SARS-CoV-2 is the causative agent of the current global pandemic, that started in December of 2109 in Wuhan, the capital of Hubei province, China. The pandemic resulted

from an animal virus that successfully infected and spread among humans; the virus has an estimated incubation time of 14–20 days, and produces a syndrome characterized by shortness of breath, sore throat and fever. This can be followed by a second stage characterized by pneumonia, that can be fatal in older people and others with either comorbidities or a genetic predisposition; however, the case fatality rate produced by this virus is not too high (at the time this manuscript was written, the World Health Organization estimated a mortality rate of 3.4%). In addition, the virus appears to have a low genetic drift, suggesting that vaccine development should provide an efficient means of epidemic control.

A number of compounds have already been used to treat coronavirus infection; in 2004, Chang-Jer et al. described that niclosamide (used as an antihelminthic drug) readily inhibited the replication of coronaviruses such as SARS-CoV (Chang-Jer et al. 2004). The similarities between SARS-CoV and SARS-CoV-2 suggest that this compound could represent a promising drug to combat the COVID-19 pandemic strain; if the treatment is successful, niclosamine could be used clinically in the near future, since the studies on its secondary effects in humans were already conducted when the drug was classified as a pharmaceutical active principle (Fig. 2). Additional small molecules, such as tetra-O-galloyl-β-D-glucose (Fig. 3) and luteolin, have also been described to inhibit the virus from adhering to human cells (Ling et al. 2004). In 1983, Higgins and coworkers successfully used intranasal treatment with interferon α to prevent experimental respiratory coronavirus infection in volunteers; interferon β was also described to inhibit SARS coronavirus infection in vitro (Tan et al. 2004). Even aminoglycoside antibiotics, such as hygromycin B, were reported to effectively control murine coronavirus infections (Macintyre et al. 1991); while the cysteine proteinase inhibitor (2S,3S)-transepoxysuccinyl-l-leucylamido-3-methylbutane ethyl ester was described to inhibit SARS-CoV Urbani strain (Yount et al. 2003). Vincent and colleagues reported in 2005 that the drug chloroquine, used since the 1940s for
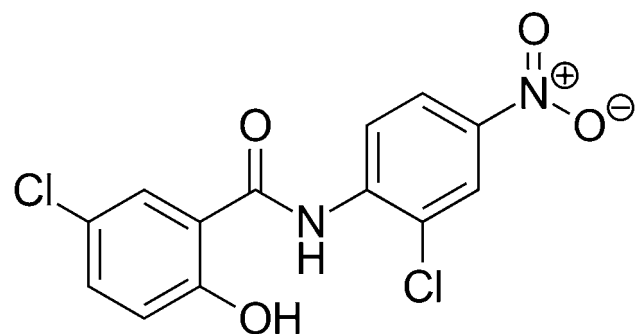
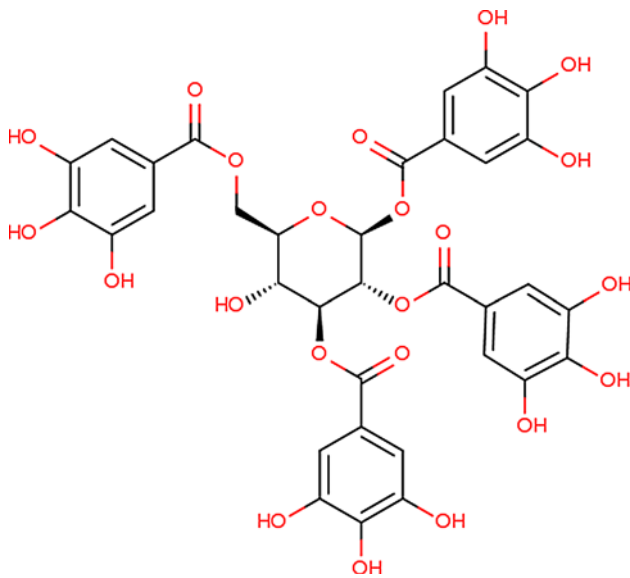**Fig.2** Chemical structure of niclosamide (2′, 5-dichloro-4′-nitrosalicylanilide)

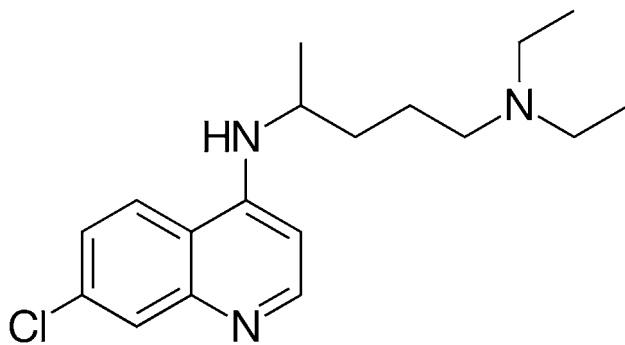**Fig. 3** Chemical structure of tetra-*O*-galloyl-β-D-glucose

Fig. 4 Chemical structure of chloroquine

prevention and treatment of malaria, strongly inhibits SARS-CoV (Fig. 4).

In addition, Veiga and coworkers proposed in 2003 an interesting way of controlling enteric coronavirus infections, the method involves *Escherichia coli* bacterial cells expressing fusions of single-chain antibodies (scFvs) to the autotransporter ß domain of the IgA protease of *Neisseria gonorrhoeae*. *E. coli* was the bacteria of choice because this organism is a common enteric commensal in healthy humans, hence the bacterium and virus share the same ecosystem.

Talbot and colleagues reported in 1988 that vaccination with a synthetic decapeptide, corresponding to a domain in the viral peplomer stalk, protected mice from a lethal encephalitis-causing coronavirus. Furthermore, Jiang et al. reported in (2005) that, vaccines based on the capsid spike (S) protein of SARS-CoV, induce strong neutralizing antibodies and protect animals from the coronavirus; in 2005,

Keng and colleagues identified amino acids 1055 to 1192, in the S2 Region of the capsid S protein, as essential to induce neutralizing antibodies against SARS-CoV.

Caliciviridae

Caliciviridae is a viral family similar to picornavirus, that exhibit a non-enveloped nucleocapsid with a diameter of 40 nm; this nucleocapsid is an icosahedral structure with a triangulation number of either 1 or 3. Caliciviruses have a non-segmented, positive-sense single-stranded RNA genome of approximately 7.5 kb; these viruses infect vertebrates, including humans, and display a novel mechanism for the initiation of protein synthesis. The capsid protein (VPg) is covalently linked to the 5′ area of the RNA and can directly interact with eukaryotic initiation factors eIF4E and eIF3; although Chaudhry et al. reported in 2006 that calicivirus RNAs appear to differ in their requirements for the proteins that integrate the translation initiation complex. The family includes 5 genera, *Lagovirus, Norovirus, Sapovirus, Nebovirus* and *Versivirus*. The genus *Versivirus* infects felines and is a typical calicivirus with an RNA genome around 7.7-kb, that is polyadenylated at the 3′ end and contains the VPg protein covalently linked to the 5′ end, as mentioned above and as is the case for picornaviruses. VPg is encoded by the open reading frame (ORF)1; the virion also contains the major capsid protein (VP1), encoded by ORF2, and VP2, a protein unique to caliciviruses that is encoded by ORF3. These proteins, that constitute important antigens for vaccine preparation, are subjected to rapid mutational rates during viral passages, and these mutations can generate strains unable to propagate. In particular, as described by Mitra et al. (2004), mutagenesis of tyrosine 24 in the VPg protein is lethal for feline calicivirus, suggesting that the tyrosine residue is essential for the formation of a covalent bond with the RNA. In addition, Sosnovtsev et al. (2005) described that, while deletion of ORF3 is lethal for the virus, smaller deletions and early terminations that preserve parts of the unique VP2 protein produce noninfectious viral particles.

The genus *Norovirus* includes the Norwalk strain, that caused an outbreak in Norwalk, Ohio, in 1968, and the Carlow variant strain, named after Carlow county, Ireland (Kearney et al. 2007). This viral genus is the most common cause of gastroenteritis and produces more than 200,000 deaths per year worldwide. Noroviruses are transmitted from person to person, mostly through contaminated waters and foodstuffs, they produce nausea, vomiting and watery diarrhea, accompanied by strong abdominal pain. In other respects, *Norovirus* is a typical calicivirus that undergoes frequent RNA mutations, with a mean evolutionary rate ranging from 1.2×10^{-2} to 1.4×10^{-2} nucleotide substitutions per site per year (Victoria et al. 2009). This ability to mutate is of great concern, as it substantially affects vaccine

effectivity; Lochridge et al. (2007) described, in murine noroviruses, that a single amino acid substitution in a crucial domain of the VP1 capsid protein is enough to evade antibody neutralization.

Astroviridae

Astroviruses were first described in 1975 as causative agents of human diarrheas, infecting both young and elderly people (Madeley and Cosgrove 1975); these viruses do not usually cause severe disease, although recent reports indicate that they can produce a neuroinvasive disease, with some infected infants developing severe encephalitis (Lum et al. 2016). The members of the *Astroviridae* family are non-enveloped viruses, with a monopartite positive-sense single-stranded RNA genome of around 7.0 kb. Their name is due to their star-like morphology, the Greek name 'astron' means star, and they have a diameter of 30 nm. The viral genomic RNA is polyadenylated at the 3′end, and contains three ORFs, known as ORF1a, ORF1b, and ORF2. ORF1 encodes the polyprotein 1ab, while ORF2 encodes the viral structural polyprotein (Chu et al. 2008); amino acid Thr227, in the capsid protein, plays an essential role in the assembly of infectious progeny viruses (Matsui et al. 2001). As is the case for other RNA viruses, astroviruses display a high evolutionary rate, estimated by Babkin and colleagues in 2012 as 3.7×10^{-3} nucleotide substitutions per site per year (Babkin et al. 2012). These viruses use a clathrin-dependent pathway to enter the host cells (Méndez et al. 2014) and, as the capsid protein is responsible for viral entry, a mutation in this viral polypeptide could result in slowing, or even fully arresting virus entry; this would render the mutant strain non-pathogenic, converting it into a good candidate for vaccine development. In (2017), Bogdanoff et al. reported that serine to proline mutations in the PL-2 epitope, located within the spike domain of the capsid protein, makes the mutated strain resistant to neutralization by a monoclonal antibody; the authors concluded that, due to the minimal changes required for an astrovirus to escape antibody neutralization, efficient vaccines must be designed to elicit a variety of antibody responses, as they would provide more extensive safeguards against viral infection.

Togaviridae

The family *Togaviridae* contains enveloped, icosahedral (with a triangulation number of 4) viruses, with monopartite positive-sense single-stranded RNA, that have arthropods, such as mosquitoes, as their natural hosts. The RNA has a methylguanosine cap at its 5′ end, which spans a non-translated 14-nucleotide long sequence, and a polyadenylated tail at its 3′end; as is the case for picornavirus, the togavirus RNA is translated into a single polyprotein of 200 kDa, that is later processed into polypeptides p150 (structural protein) and p90 (RNA-dependent RNA polymerase).

The family includes two genera, *Alphavirus* and *Rubivirus*, that cause human diseases such as German measles (aka rubella); some examples are Sindbis virus, that produces arthritic disease, and Chikungunya virus, that causes joint pain (arthralgia) (Chen et al. 2018). The study of the mutational rate of these viruses is important, as it affects the effectivity of human vaccines. The vaccine currently used against German measles is a live attenuated version of the virus; this inoculation is particularly important in women of childbearing age, as viral infection during early pregnancy can produce serious consequences, such as miscarriages, still births and severe birth defects in the child, denominated congenital rubella syndrome. Alterations in the rubella virus genome can result in point mutations, but they can also generate defective-interfering RNAs, containing a reduced number of nucleotides, hence, considerably decreasing the virus titer; some of these RNAs can span 7000 nt, although they are usually shorter than 300 nt (Derdeyn and Frey 1995). Where attenuated vaccines are used, as is the case for rubella, new mutations can either prevent reversion or facilitate it. In addition, the mutated vaccine can display adverse reactions, either when used as part of a multi-virus vaccine or when applied alone; one such example, reported by Domegan and Atkins in (2002), involved cellular apoptosis in rat glial cell cultures. The La polypeptide is a 47 kDa protein that can act as an autoantigen in human autoimmune diseases, such as systemic lupus erythematosus and Sjögren syndrome. This protein was found to interact with the 5′stem loop RNA of the rubella virus; in some cases, this interaction can affect rubella pathology, as well as produce adverse reactions when vaccinated against this virus, although the benefits of rubella vaccination clearly outweigh the risks. Mutations in the 5′ stem loop result in a significant reduction in the production of viral non-structural proteins (Pugachev and Frey 1998). As togaviruses are enveloped viruses, mutations in their glycosylated membrane proteins, E1 and E2 that form a heterodimer, could prevent the viral membrane fusing with the cell membrane; Yang and co-workers reported in 1998 that amino acid substitutions, at positions 81 and 109 in the E1 protein, disrupted heterodimer formation between E1 and E2, resulting in the virus lacking membrane fusion activity. Additional mutations in the E1 protein, such as the substitution of a single tyrosine residue in the glycoprotein reported by Yao and Gillam (2000), was sufficient to block virus release. In an interesting publication in 2001, Kakizawa et al. discovered 21 different mutations present in an attenuated strain of rubella virus, as compared to the wild-type strain; 13 of them were in the non-structural protein, while 5 affected the structural protein and three occurred in the untranslated region. A critical finding

reported by the authors is that, the mutations that cause viral attenuation, appear to be different in each vaccine strain.

Furthermore, the GDD motif present in the p90 protein is important for RNA replication, since alteration of either of the aspartate residues stops virus replication; but point mutations could also fully restore the attenuated virus, resulting in the production of infectious virions, rendering the vaccines unsafe. Attenuation of rubella strains often results in temperature sensitive mutants, with mutation of histidine at position 1042 in the p150 region, essential for generation of the phenotype (Sakata et al. 2009). Additional side effects produced by the rubella vaccine could relate to the ability of the capsid protein to interact with mitochondria, inhibiting protein import into the organelle; this matter needs to be addressed to eliminate this unwanted effect when designing vaccines (Ilkow et al. 2010).

Viruses containing negative-sense ssRNA

Bunyaviruses

Bunyaviruses have been recently reclassified as an order, containing the families *Hantaviridae, Feraviridae, Fimoviridae, Jonviridae, Nairoviridae, Peribunyaviridae, Phasmaviridae, Phenuiviridae,* and *Tospoviridae*. They are enveloped negative-sense ssRNA viruses, filiform in shape, that commonly infect in arthropods, although some species are human pathogens; the latter include viruses such as the Crimean-Congo hemorrhagic fever, Rift Valley fever (with a fatality rate ranging from 0.5 to 2%) and hantavirus hemorrhagic fever (with a mortality rate that can reach 55%). Their genomic RNA is tripartite, formed by an L segment that encodes an RNA-dependent RNA polymerase, an M segment that codes for the viral glycoproteins, and a S segment that encodes the nucleocapsid protein. There are currently no licensed vaccines for bunyaviruses, although vaccine strain MP-12, as well as non-spreading replicon particles from the Rift Valley fever virus, remain as vaccine candidates; this means that, at this stage, it is not possible to evaluate the effect of mutations on the attenuated viruses, that could revert the vaccine strain into fully pathogenic virions. However, some authors have described reverse genetic approaches, that involve selectively disrupting the viral deubiquitinase and deISGylase enzymes, to generate viral strains with putative use both in therapy and as vaccines (Scholte et al. 2017). However, treatment of bunyavirus diseases mainly relies on antivirals, such as ribavirin, with good in vitro activity, but less in vivo efficacy; other antivirals include favipiravir, that protects from hepatitis but not from encephalitis, and the nucleoside analog BCX4430 that, at least in mice, increases survival (Atkins and Freiberg 2017). Future treatments could take advantage of the ability of endosymbiont bacteria, such as *Wolbachia*, to inhibit the replication cycle of bunyaviruses, and this microorganism might even eliminate the virions (Schnettler et al. 2016). Naturally, if *Wolbachia* is used as antiviral treatment, mutations affecting the bacterium could affect the efficacy of the treatment.

Rhabdoviruses and filoviruses

Rhabdoviruses and filoviruses belong to the *Mononegavirales*, an order that contains a large number of viruses with non-segmented negative-sense ssRNA. In addition to the rabies and Ebola viruses, this group includes the human respiratory syncytial virus, measles, and mumps viruses, all of which cause significant disease and suffering in humans. Vesicular Stomatitis Virus (VSV) is the rhabdovirus archetype, and Wang et al. (2015) used this virus to develop a recombinant attenuated strain that could represent a good antigen delivery platform to vaccinate humans against other *Mononegavirales*.

Rhabdoviruses are bullet-shaped enveloped viruses, that are approximately 75 nm wide and 180 nm long, with a helical capsid and unipartite negative-sense ssRNA, they belong to the genus *Lyssavirus*. The mature virions contain 5 proteins, two of these (L and P) constitute the polymerase complex, the glycoprotein (G) makes the peplomers, the nucleoprotein (N) is the major nucleocapsid component, and the matrix protein (M) surrounds the nucleocapsid. The G glycoprotein, that makes trimers and projects, like spikes, from the nucleocapsid, is responsible for binding to receptors on the host cells, and is the protein that induces neutralizing antibodies (Wiktor et al. 1973). Although some rhabdoviruses, such as vesicular stomatitis virus, can induce interferon production, a cytokine shown to offer animals protection against viral progression (Youngner and Wertz 1968), defense against viruses like rabies is best achieved by vaccination with attenuated viral strains. The complete nucleotide sequence of the attenuated rabies virus SAD B19 was reported by Conzelmann et al. (1990); but it was not until 2008 that Szanto and colleagues published the complete sequence of the raccoon rabies virus, the second North American wildlife rabies virus strain that was fully characterized.

Rhabdoviruses exhibit an exponential increase in fitness during large populations transmission (fitness is a parameter that measures the virus capability to replicate) and this increase can reach 5000% after 50 passages; in the case of bacteria, such as *E. coli*, fitness increases are hyperbolic and only reach a maximum of 37% after 2000 generations (Lenski and Travisano 1994; Novella et al. 1995). Single nucleotide mutations are sufficient to affect the degree of fitness in RNA virions (Sanjuán et al. 2004a) and even silent mutations can significantly contribute to adaptation in these viruses (Novella et al. 2004, 2005). Novella et al. described

in (2010) that, at a given number of viral passages, control populations underwent bigger fitness gains than mutant populations. In addition, the A-to-I deaminase enzymes (ADARs), that perform RNA editing on viral negative-sense double-stranded RNA, change adenosines to inosines (that are read as guanosines) adding to the mutation rate; Carpenter et al. (2009) described hypermutations in the sigma virus, a rhabdovirus that infects *Drosophila melanogaster*, generating protein diversity in the central nervous system of the insect.

It is difficult to ascertain the mutations occurring in rabies virus, as much of our knowledge comes from VSV studies; relevant mutations for the latter include those reported by Pringle in 1970, caused by 5-fluorouracil, 5-azacytidine and ethyl methane sulfonate, and the spontaneous thermo-sensitive mutants discovered by Flamand, also in (1970). The pioneering work of Clark and Koprowski (1971) identified generation of "fixed" rabies virus mutants by treatment with HNO$_2$, 5-fluorouracil, or 5-azacytidine. Some of these mutants originated are thermosensitive (with a non-permissive temperature of 40.5 °C) and exhibit a remarkably low incidence of either reversion or leakiness, making them good candidates for putative vaccines. The characterization of low-reversion rabies virus mutants is indeed important, as Clark (1980) reported that, serial propagation of attenuated rabies strains at a low multiplicity of infection, resulted in the appearance of virulent revertants, probably due to selective replication enhancement of these variants during virus culture.

The first stage of infection for all enveloped viruses involves fusion between the virus envelope and the membrane of the target cell; this process is mediated by the transmembrane protein G in rhabdoviruses, as mentioned above. The mature G protein associates in trimers (Whitt et al. 1991; Gaudin et al. 1992) and acquires this configuration in the endoplasmic reticulum; the putative fusion peptide for rabies virus is located between amino acids 102 and 179 (Durrer et al. 1995). Other roles for this glycoprotein are its involvement in humoral and cellular immune responses, and its role as the first determinant for neurotropism (Badrane et al. 2001).

Seif and collaborators demonstrated in 1985 that mutations in the capsid glycoprotein G were essential to obtain either attenuated or nonpathogenic strains of rabies virus; in particular, a point mutation at position 333, in which arginine was replaced by either glutamine or glycine, proved crucial to generate a phenotype that is avirulent in mice. Four years later, Tuffereau et al. (1989) confirmed that Arginine 333 is absolutely essential for pathogenicity in the "fixed" rhabdoviruses CVS and ERA strains, although the viruses could revert to full pathogenicity by mutations that restore arginine at position 333 (Takayama et al. 2006). Fortunately, it appears that the nonpathogenic glycoprotein gene

is dominant over the pathogenic gene (Faber et al. 2007). Furthermore, Ito et al. had already described in 1994 a non-pathogenic mutant of rabies virus, in which the arginine at position 333 was preserved, but contained other mutations, such as D instead of G at position 255, that rendered the strain nonpathogenic. However, after substitutions such as 333H, 333 N, or 333Q, in rabies virus street strains isolated from non-hematophagous bats in Brazil, the virions remained pathogenic for adult mice (Sato et al. 2009); these results confirm the crucial role played by the amino acid at this position in viral pathogenicity. It was originally thought that virus strains lacking the G protein would not be able to be released, by budding, from infected cells; Mebatsion et al. reported in (1996) that, although G protein is not absolutely essential for budding, this polypeptide produced a sixfold increase in virus productivity. In addition, Faber et al. discovered in (2005) that, a single amino acid change (Asn to Lys at position 194) in rabies virus glycoprotein G, increases virus spread and significantly enhances virus pathogenicity.

Mutations in the G protein gene, particularly those inducing changes between amino acids 118 and 139, are important for the membrane fusion activity of the protein and, therefore, interfere with the viral replication cycle; this is an important consideration for vaccine design (Fredericksen and Whitt 1995). Indeed, and according to Shokralla et al. (1998), mutations that alter the carboxy-terminal region of the G glycoprotein, affect membrane fusion activity; in particular, substitutions of Gly 395, Gly 404, Gly 406, Asp 409, and Asp 411 with Glu, Ala, Ala, Asn, and Asn, respectively, decrease virion-cell fusion efficiency. Furthermore, substitution of a single glycine residue in the transmembrane domain of G protein is enough to abolish the fusion activity of the virus (Cleverley and Lenard 1998). Shokralla et al. (1999) used site-directed mutagenesis to generate double-site mutations in the G protein of VSV; these mutants could represent appropriate attenuated strains for vaccine development, although, even dual mutants could revert to full infectivity, albeit at a very low rate. In 2002, Langevin and Tuffereau discovered novel mutations in rabies virus that mapped outside the areas thus far identified as antigenic, they included a serine or a valine residue replacing a phenylalanine at position 318, and a tyrosine or an arginine replacing a histidine residue at position 352; in both cases, the mutations conferred resistance to neutralization by the neurotrophin receptor p75NTR. Alto et al. (2013) studied the evolution of VSV genomic RNA, in environments either under constant or fluctuating temperatures, and reported that, under any of the environmental conditions tested, the majority of genome substitutions occurred in the G glycoprotein gene, suggesting the importance of this polypeptide in rhabdovirus adaptation to changing thermal conditions.

Mutations in the L– polymerase complex and the leader-N gene junction are also important in vaccine design, since

attenuated vaccines containing mutations in these genes can revert to a quasi-wild-type, or even to a wild-type, and undergo a productive pathologic cycle. Accordingly, Chuang et al. (1997) studied the switch from transcription to genome replication in VSV polR mutants, that are defective in RNA replication, and concluded that the L protein contains second-site suppressors for polR, hence switching the RNA polymerase into replication mode to generate virions.

The matrix protein (M) is involved in the suppression of β interferon gene expression, this is achieved through a general inhibition of both RNA and protein synthesis in the infected cell (Ahmed et al. 2003). Accordingly, any mutation in M that abolishes this ability would, at least in principle, facilitate virus neutralization by the host interferon system.

The matrix protein gene represents a good target for mutational studies, since this polypeptide targets neural mitochondria inducing cellular apoptosis (Gholami et al. 2008); hence, a mutated M unable to suppress β interferon gene expression or induce cellular apoptosis, would be a good candidate to incorporate into the design of attenuated rhabdovirus vaccines. The M proteins of these viruses also control intracellular virus accumulation, which is a crucial factor in disease development; Finke et al. (2010) constructed chimeric rabies viruses, with their M proteins replaced by lyssavirus M protein, and confirmed the role of M proteins in intracellular virus accumulation. More recently, Ke et al. (2017) reported that the M protein of the hemorrhagic septicemia virus plays a role in the suppression of host transcription, this includes suppression of the interferon response and the antiviral gene expression.

Mutations in the rhabdovirus N protein are also valuable; Masatani et al. described in (2011) that the amino acids located at positions 273 and 394, in rabies virus N protein, are important not only for pathogenicity, but also for evading retinoic acid-inducible gene I (RIG-I)-mediated innate immunity.

Another major consideration is the fact that, an increase in the rate of N-glycosylation in the G protein (the common N-glycosylation sites are Asn-37 and Asn-319, with an additional site at A-247), can occasionally result in the generation of attenuated rhabdovirus strains (Yamada et al. 2012). The authors reported that serial passage of a street rabies virus in mouse neuroblastoma cells produced strains with lower pathogenicity, due to the increased glycosylation described above, making them effective attenuated strains. Yamada et al. reported in (2014) that efficient N-glycosylation at position 37, but not at position 146, in the street rabies virus glycoprotein, resulted in reduced viral pathogenicity. Nevertheless, to avoid the risk posed by mutation reversion, it is preferable to use vaccine strains containing multiple mutations, in the genes coding for proteins G, N and M, as opposed to single mutations (Nakagawa et al. 2012); although there is always the danger of accumulating

multiple mutations, for instance by serial passages in BHK cells, that could render the attenuated rabies viruses hypervirulent (Virojanapirom et al. 2012).

Mutations in the L protein of rhabdoviruses appear to also be important; Tian et al. (2015) reported that, amino acids K1685 and K1829 in the L protein, are involved in pathogenicity and viral immune evasion, indicating a potential use for mutans in these residues in both therapeutics and as a tool to produce avirulent rabies vaccines. Additionally, mutations in the highly conserved region of the protein, corresponding to position 1914–1933 (NPYNE), have pleiotropic effects on the virus biology, according to Nakagawa et al. (2017); the authors suggested that these mutants could be useful in the design of attenuated vaccines.

Epistasis, the interaction between genes, is also a crucial topic that plays a major role in how different mutations affect rhabdovirus fitness. This subject was addressed by Sanjuán et al. in their (2004b) publication; the authors generated 47 mutated VSV genotypes and compared the compensatory adaptation for virions with either one or two deleterious mutations. They reported that several pairs of mutations exhibited significant interactions that affected viral fitness; these include antagonistic (a pair of mutations that together create a smaller effect than the sum of the two individual effects) and synergistic epistasis (when the effect on fitness of the two mutations together is bigger than the sum of the two individual mutations). In a later publication Sanjuán and Elena (2006) concluded that, while simpler RNA genomes usually exhibit antagonistic epistasis, more complex genomes tend to display synergism.

A number of antivirals against rhabdovirus have been described, these include highly sulfated K5 *E. coli* polysaccharide derivatives (Cagno et al. 2014), that effectively inhibit human infection by respiratory syncytial virus (Villa et al. 2017); Chattopadhyay described in (2006) that a small cationic protein, from the egg white of the turtle *Caretta caretta*, is an efficient antiviral compound against rhabdoviruses (Chattopadhyay et al. 2006). Naturally, the antiviral effectiveness of these molecules is affected by viral mutations that could render these viruses resistant to them.

Ebolavirus is probably the best-known member of the *Filoviridae* family, other members include Marburg virus and *Cuevavirus*; the latter, recently labeled as Lloviu virus, was named after a cave in Asturias, Spain, by Negredo et al. (2011). Filovirus cause severe, mainly fatal, hemorrhagic fever in humans. The *Filoviridae* family includes two classic genera, *Marburgvirus*, with several strains, and *Ebolavirus*, that encompasses six species, Sudan, Zaire, Ivory Coast, Reston (first Ebolavirus in mammals other than primates), Bundibugyo (Towner et al. 2008), with the above mentioned *Cuevavirus* as the most recent addition (Negredo et al. 2011). It is conceivable that new genera will be added to the filovirus group in the near future, as new animals

and ecosystems are studied, due to the genetic drift of these viruses. As it is widely known, *Marburgvirus* was described in 1967, in Marburg, Germany, during an accidental laboratory outbreak; while *Ebolavirus* was detected in 1976 in Yambuku, Zaire, and displays a mortality rate that can reach 90%.

These viruses contain long cylindrical/tubular nucleocapsids (up to 1000 nm long), that appear to display knots on their surfaces, an appearance typical of these viruses when examined under the electron microscope. The genome is monopartite, with negative-sense single-stranded RNA of approximately 19,000 nucleotides; the genome encodes seven structural proteins and is organized: 3′–leader–NP (nucleoprotein)–VP35 (component of the viral RNA polymerase complex, it constitutes a viral assembly factor that also inhibits host interferon production)–VP40 (matrix protein that penetrates the plasma membrane)–GP/sGP (glycoprotein that forms 7–10 nm long spikes)–VP30 (RNA binding protein, acts as a transcription activator)–VP24 (multifunctional secondary matrix protein)–L (RNA polymerase)–trailer–5′. In contrast to rabies vaccine, that has been available from the time of Louis Pasteur, in the nineteenth century, the vaccine for Ebolavirus only became available in December 2016; this is a recombinant vesicular stomatitis virus-Ebola vaccine, approved for medical use in the European Union and United States in 2019. Earlier vaccines proved successful in non-human primates; they contained either adenovirus vectors encoding modified Ebola GP polypeptides (Sullivan et al. 2006), or Ebola strains lacking VP30 (Halfmann et al. 2009). Lázaro-Frías and colleagues reported in 2018 a new strategy for vaccines against *Ebolavirus*, the authors proposed the use of poxviruses expressing GP and VP40 antigens.

Ebola viruses display high mutation rates, in the order of 2.0×10^{-3} substitutions per site per year (Jenkins et al. 2002); of particular interest are those mutations that affect surface glycoproteins, which interact with the two viral receptors, Niemann–Pick Type C1 (Carette et al. 2011) and TIM-1 receptors (Kondratowicz et al. 2011; Yuan et al. 2015). Matassov et al. (2015) highlighted the importance of these glycoproteins in immunization; they used a recombinant vesicular stomatitis virus with its glycoprotein removed and replaced by the Ebola GP polypeptides. In addition, Lennemann and coworkers, also in 2015, studied the role of mutations on the glycoprotein GP2, and found that some of the mutations arrested virus fusion to the host membrane. Once the virus is internalized, glycoprotein GP is processed by the proprotein convertase furin, originating the proteolytic products GP1 and GP2 essential for Ebola virus pathogenicity (Volchkov et al. 1998). Watanabe and colleagues demonstrated, in 2000, that the coiled-coil motif present in GP2 plays an important role in facilitating the entry of Ebola virus into the host cells. The GP glycoprotein

contains a highly conserved hydrophobic region (encompassing residues 524–539) and mutations in that region, involving amino acid substitutions such as I532R, F535R, G536A, and P537R, almost completely abolish infectivity (Ito et al. 1999). In addition, mutations of the two phenylalanine residues, found in two linear domains of GP1, are critical for viral cell entry; this, in turn, provides a novel target for vaccines and antiviral therapies (Mpanju et al. 2006). The phenylalanine at position 88 appears to be most important for host cell entry, while the residue at position 159 plays a less relevant role, as it is only indirectly involved (Ou et al. 2010). It is important to consider, however, that mutations in the envelope glycoproteins of Ebola viruses occasionally only restrict viral entry in a host species-specific manner, as reported by Martínez and colleagues in 2013. Furthermore, a single alanine-to-valine amino acid substitution in the surface glycoprotein, A82V mutation, can render viral strains more pathogenic to humans; the Ebola epidemic of 2013–2016, caused by one such strain, infected more than 28,000 people, more than the combination of all previous outbreaks, and resulted in 11,000 casualties (Diehl et al. 2016, Bedford and Malik 2016). Additional mutations in GP, such as amino acid substitutions R29V, T206M and T230A, reported by Urbanowicz et al. (2016), revealed that those mutations provided additional increases in primate-specific infectivity.

Prins et al. (2010) studied the effect of mutation in VP35, they introduced two amino acid substitutions, K319A and R322A, in the protein that completely abrogated the VP35 dsRNA binding activity; this changes prevented the Ebola virus from infecting guinea pigs, highlighting the importance of these type of strains in vaccine development.

Nevertheless, it is important to remember the statement by Olabode et al. (2015) indicating that "Ebola virus is evolving but not changing", as no functional changes have been identified in the different outbreaks of the disease, from 1976 to 2014.

Orthomyxovirus

The *Orthomyxoviridae* family contains ssRNA of negative polarity, usually composed of six to eight polyadenylated segments, enclosed in a helical capsid; they are surrounded by a membrane from which two polypeptides protrude, one of the proteins displays enzymatic activity, while the other interacts with red cells (neuraminidase, and hemagglutinin respectively). Both hemagglutinin and neuraminidase are immunologically independent components of the viral surface (Seto and Rott 1966), and serological tests indicated that the surface antigens in group A influenza virus display a wide antigenic variation (Pereira 1967; Tůmová and Pereira 1968; Pereira 1969; Schild and Newman 1969). It soon became apparent that the neuraminidase of certain avian

influenza A strains was closely related to the enzyme present in human influenza virusesSchild and Newman described in 1969 that the neuraminidase of particular avian influenza strains, including A/Turkey/Massachusetts/65, was antigenically similar to the enzyme present in human strains; these results were confirmed by Easterday et al. (1969), that carried out inhibition and immunoprecipitation tests. Webster and Pereira had already described in (1968) that influenza viruses from human and avian sources shared a common surface antigen. This close relationship between the neuraminidase enzyme in human and avian viruses suggests a genetic interaction, in nature, between influenza strains from different hosts, resulting in the antigen radiation observed in the strains circulating around the world.

The *Orthomyxoviridae* family includes four genera, *Thogotovirus*, *Isavirus, Quaranjavirus* and *Influenzavirus*; the latter is the best known, due to its relevance to human health, and is classified into four groups, A, B, C, and D. *Thogotovirus* was originally classified as part of the old *Arboviridae* family, with hosts among both vertebrates and invertebrates (Albanese et al. 1972). *Isavirus* (Hellebø et al. 2004) infects mainly euryhaline fishes belonging to the genus *Salmo* (i.e. *S. salar*); while *Quaranjavirus*, with *Quaranfil* virus as the type species, belongs to a family branch highly divergent from the rest of orthomyxovirus, and was isolated from *Rhipicephalus* ticks (Choletti et al. 2018). The prominent genus, *Influenza*, produces regular pandemics in humans, that involve groups B and C, but mainly group A; the latter undergoes frequent antigenic shifts, requiring annual vaccination against the seasonal strain, making this group the most likely to produce a world pandemic (Gasparini et al. 2014). Group D is the most recently discovered, and was isolated from pigs and cattle with respiratory syndromes (Hause et al. 2014; Collin et al. 2015; Barberis et al. 2016).

No one currently questions the fact that influenza is caused by a virus, but this was not the case at the end of the nineteenth century, or even during the first two decades of the twentieth century, when it was erroneously assumed that the illness was caused by a bacterium, named *Bacillus influenzae* (Twort and Twort 1921). Additional misconceptions about the etiological agents of other viral diseases are described in the review by Villa et al. (2018).

The incorrectly named, infamous "Spanish flu" (January 1918–December 1920) was caused by the Influenza A pandemic strain N1H1, an orthomyxovirus containing genes of avian origin, that infected over 500 million people a caused an estimated 50 to 100 million casualties (3–6% of the human population at the time). This was the second known flu pandemic to spread over the world (Villa et al. 2018); the first well documented worldwide occurred in 1580, it started in Asia and spread to Europe, via Asia Minor, and North–West Africa, eventually reaching the Americas

(Potter 2001). More recent pandemic waves include the "Asian influenza", this was originated by an avian H2N2 virus that started in China in 1957 and caused an estimated two million deaths worldwide; the "Hong Kong" influenza of 1968, produced by a H3N2 influenza A virus that resulted in one million deaths; while a new strain of N1H1 emerged in the 2009 pandemic with the ability to infect, not only humans, but also several animal species, such as pigs, horses and birds. Barberis and co-workers published in (2016) an interesting review on the history of influenza. Although the influenza virus displays a tendency to create pandemics, this is not the case for other members of the *Orthomyxoviridae* family.

Pandemic waves are not only produced by viruses, but can also result from bacterial infections; this is the case for the "black death", caused by the bacterium *Yersinia pestis* and known as bubonic plague, that produced several infection waves and resulted in the most lethal pandemic in the history of humanity. There are a number of pandemics, caused by this bacterium, recorded in human history, these include the Justinian plague in the sixth century, that killed an estimated 20–50 million people; the middle ages plague in the fourteenth century, that ravaged Europe and caused an estimated 75–200 million deaths, representing more than a third of the European population of the time. The last bubonic pandemic originated in China, in the middle of the nineteenth century, and rapidly spread to the American continent, due to shipping harbor traffic; the World Health Organization did not declare that this pandemic had ended until 1959, although there was a further outbreak in India, in 1994, that caused 50 deaths.

Virologists identified a continuous genetic variation in Influenza viruses, not present in other viral families such as Rhabdovirus or Coronavirus, that causes an antigenic drift resulting in the variation in the viral surface antigens; this antigenic variation is responsible for the flu virus evading the immunity caused by vaccination with the previous year pathogenic strain, hence requiring an effective vaccine to be updated annually. Although scientist have renewed their efforts to attain an "universal influenza vaccine", this matter still remains a challenge (Wiersma et al. 2015; Barberis et al. 2016). According to Barberis and colleagues "current research priorities include the development of a universal influenza vaccine that could offer protection against all influenza virus strains, thereby overcoming the challenges faced due to antigenic drift and shift or of co-circulation of different viral strains". Already in (1973), Kendal and Kiley published an article on the antigenic divergence between the neuraminidases of Asian and Hong Kong influenza viruses, and associated this divergence to short changes in the primary structure of the neuraminidase; but the full complexity of this matter did not become apparent until it was discovered that influenza viruses of different origin could

recombinate to either originate completely new viral strains (Desselberger et al. 1978), or at least increase their genetic variation (Young and Palese 1979).

Paramyxoviruses

The best-known members of the *Paramyxoviridae* family are the causative agents of measles and mumps; they are highly contagious, enveloped viruses containing negative-sense single-stranded RNA, that belong to the genus *Morbilivirus* and *Rubulavirus*, respectively. The natural hosts of these viruses are humans, and no other animal reservoir has been thus far described. The measles virus was originally isolated in 1954, and the World Health Organization currently recognizes 24 different strains of this virus, belonging to eight clades named A, B, C, D, E, F, G, and H. The first effective measles vaccine was developed by Maurice Hilleman, licensed in 1963 and estimated to prevent over one million deaths per year; although recent data indicates that measles virus is still responsible for over 100,000 fatalities every year. Measles is considered eradicated in some developed countries, but still infects many people in non-developed nations (Shi et al. 2011). As is the case for other pathogenic viruses, it is essential to identify the mutation rate of the viral strains, not only for vaccine development, but also to estimate the probability of antiviral-resistant populations arising. The measles virion displays mutation rates similar to other ssRNA viruses in the laboratory, with an estimated mutation rate of 1.43 per replication (Schrag et al. 1999), while the virus exhibits high genetic stability in nature (Rima et al. 1997); this different viral behavior in the two environments remains unexplained.

Nichols (1963) and Nichols et al. (1965) reported in their publications that growth of the measles virus caused in vitro chromosome damage (mainly breakages); these chromosomal abnormalities were also reported as a result of measles vaccination (Levenshtam et al. 1969). The Edmonston strain of measles virus was described to cause polyokaryocytosis, in which the polykaryocyte nuclei tend to fuse (Heneen et al. 1970); while in (1971), Parker et al. reported that vaccination with an attenuated measles virus strain could produce an infection in the human central nervous system. Despite these serious side effects described above for the vaccine, Mina et al. (2019) reported that it remains crucial to continue widespread vaccination against measles because, in addition to the deaths directly caused by the virus, measles infection results in major detrimental effects in the individuals that survive. These authors concluded that infection by this virus induces a progressive loss of preexisting humoral antibodies in the patient, hence eliminating the protection the immune memory exerted against a variety of diseases, including influenza. This results, taken together with recent data indicating that measles infections cause prolonged immunosuppression in unvaccinated children (Petrova et al.

2019), in addition to the described ability of the measles vaccine to enhance immunological resistance to a variety of bacterial and virus pathogens, highlight the importance of maintaining worldwide measles vaccination programs.

It has been proposed that the measles virus is a paradigm of viral stability in the field, as the antigenic composition of the vaccine used to prevent it has remained efficient since it was developed, in the 1960s, and confers protection against the 24 circulating genotypes (Muñoz-Alía et al. 2017). These authors also reported a new D4 subgenotype (D4.2) of the measles virus, suggesting that, although genomic drift occurs, it appears to happen at a much lower rate in measles than in members of the *Orthomyxoviridae* family. The new subgenotype represents a distant evolutionary relative in the D4 genotype and, as opposed to other measles strains, is distinguishable by neutralization with antibodies raised against the viral epitope NE. Some virus variants of the H clade in China appear to be undergoing similar developments (Shi et al. 2011). In conclusion and, despite the genetic stability of the measles virions, it is likely that in the future there will be a need to update the vaccines currently used around the world to combat this disease.

The mumps virus, as indicated above, is the second classic example of a paramyxovirus; the disease it causes has long been known to mainly affect children, although it can cause severe symptoms in adults, including orchitis that can lead to male infertility. The name "mumps" is the common name given to the disease in places like England, but it is known as "brank" in Scotland, although medical practitioners prefer the denomination "angina maxillaris", in particular after Hamilton's publication in 1790. The disease is characterized by the painful swelling of one or both parotid salivary glands and, although the mortality rate is low for this disease, patients can develop side effects such as benign torticollis (McGavin 1913), meningitis (Wollstein 1921), including fatal meningitis (Griebel and Schnee 1950), pancreatitis, inflammation of the heart (Rosenberg, 1945), permanent deafness (Barr 1889), grave diabetes mellitus and pulmonary tuberculosis (Gilhespy and Holden 1917), as well as the orchitis mentioned above (Burne 1851; Hviid et al. 2008). The disease is currently prevented, in developed countries, by a vaccine, either on its own or in combination with rubella and measles; Maurice Hilleman is responsible for the development of over 40 vaccines, including measles and mumps (Buynak et al. 1969). The current vaccine has been described to only fully protect around 80% of the vaccinated population (Schlegel et al. 1999), with the remaining 20% only partially protected.

The mumps virus genomic RNA is negative-sense and single-stranded, it contains 15,384 bases that encode nine proteins, and displays low genetic drift; the latter is supported by the fact that the vaccine against this virus has been efficient for so many years. The World Health Organization

recognized in 2015 twelve genotypes for the mumps virus (from A to N, excluding E and M), based on the nucleotide sequences of two genes, a small hydrophobic (SH) and the hemagglutinin-neuraminidase (Jin et al. 2015). After sequencing a variety of disease-causing mumps viruses, it was discovered that the degree of genetic divergence (heterogeneity) for the small hydrophobic gene is 20%, while it is only 9% for the hemagglutinin-neuraminidase gene. This genomic drift is consistent with the field data on the effectiveness of the currently used vaccines. However, routine monitoring of the viral genetic variation, in both measles and mumps, is essential to improve the molecular diagnostics of the viruses; this can be easily and rapidly achieved by next-generation sequencing (Dilcher et al. 2018). Using this technique, Willocks et al. (2017) demonstrated in Scotland that, an outbreak of mumps in the student population with high vaccination rates, was caused by a viral strain containing a genetic variation; this again highlights the need for vigilance in the health authorities, to update and maintain the effectiveness of vaccines. A classic example of this is the Urabe AM9 mumps vaccine, consisting of a mixture of viruses with different amino acids at position 335 in the hemagglutinin-neuraminidase (Brown et al. 1996), that displayed differences in attenuation rates when tested in the rat neurovirulence model (Shah et al. 2009; Schinkel et al. 2017).

Concluding remarks

RNA viruses exhibit higher mutation rates that DNA viruses, this allows for fast evolution and, accordingly, these viruses can develop antiviral resistance and overcome vaccines much more rapidly than the DNA viruses. This also translates into the fact that pathogenic RNA viruses, that cause many diseases and deaths in humans, represent the major viral group involved in zoonotic disease transmission, and are responsible for worldwide pandemics. In general, the fact that RNA viruses display greater substitution rates than their DNA counterparts is mainly due the low fidelity of the RNA polymerases, which usually lack a proofreading mechanism and have high mutation rates. This lack of replication fidelity, together with the fact that RNA viruses can undergo spontaneous mutations, results in genetic variants displaying different viral morphogenesis and variation on their surface glycoproteins, that affect the viral antigenicity and can result in the production of viral quasispecies (Mandary et al. 2019).

The above mentioned low replicative fidelity and population diversity directly affects fitness and virulence in RNA viruses. Viral RNA-dependent RNA polymerases display a high frequency of ribonucleotide

misincorporation, that contributes to genetic diversity; the speed at which RNA viruses create genetic variants, allows the viral population to quickly adapt to changing environments, even during host infection. Fitzsimmons and coworkers suggested that speed is more important than accuracy, adding that RNA viruses prioritize speed over fidelity, hence resulting in high mutation rates, as it is hard to concomitantly achieve both speed and accuracy (Fitzsimmons et al. 2018). Mathematical models for population genetics identified what they term as "extent of genetic diversity", this is a theoretical calculation of the maximum number of mutations a haploid genome can withstand when the yield of offspring (fecundity) is fixed. These studies led researchers to believe that RNA viruses display the highest bearable mutation frequency, but this matter has only been tested in vitro with chemical mutagenesis (Korboukh et al. 2014). Lauring et al. (2012) studied the effect of mutagenesis on virus evolvability and pathogenesis, by comparing the poliovirus wild-type strain to two synthetic viruses engineered to contain large-scale synonymous mutations on their capsids. Remarkably, the authors discovered that, although the two engineered viruses encoded a nucleocapsid identical to that of the wild-type virus, the mutations caused a reduction in the robustness of the virion, with one of the mutants behaving as an attenuated virus. It also appears that the location of the mutated nucleotide affects the capability of viral mutation. These two facts, taken together, could represent a new era in the design of novel live attenuated vaccines, with the candidate strains arising by molecular engineering, and including a restricted evolutionary capacity in their design. Lauring et al. (2012) also reported that the lack of replication fidelity favors RNA viruses, because it creates a population that contains individual variants, hence, facilitating adaptation to changes within the host it infects. This quasispecies quality of RNA viruses also promotes the appearance for zoonotic virus, that regularly cause human pandemics with considerable fatality rates.

The quasispecies phenomenon could also be responsible for the differences in virulence, as well as plaque size and morphology, found in RNA viruses, and these studies are important to determine if severe infections are caused by a single strain or are the result of the cooperative effect of viral quasispecies, displaying different mutations. This information would give an important insight into virus evolution and adaptation, as well as positively contributing to the design of more effective, and longer-lasting, vaccines against RNA viruses (Mandary et al. 2019).

Compliance with ethical standards

References

Ahmed M, McKenzie MO, Puckett S, Hojnacki M, Poliquin L, Lyles DS (2003) Ability of the matrix protein of vesicular stomatitis virus to suppress beta interferon gene expression is genetically correlated with the inhibition of host RNA and protein synthesis. J Virol 77:4646–4657

Albanese M, Bruno-Smiraglia C, Di Cuonzo G, Lavagnino A, Srihongse S (1972) Isolation of *Thogoto* virus from *Rhipicephalus bursa* ticks in Western Sicily. Acta Virol 16:267

Alto BW, Wasik BR, Morales NM, Turner PE (2013) Stochastic temperatures impede RNA virus adaptation. Evolution 67:969–979

Atkins C, Freiberg AN (2017) Recent advances in the development of antiviral therapeutics for Rift Valley fever virus infection. Future Virol 12:651–665

Babkin IV, Tikunov AY, Zhirakovskaia EV, Netesov SV, Tikunova NV (2012) High evolutionary rate of human astrovirus. Infect Genet Evol 12:435–442

Badrane H, Bahloul C, Perrin P, Tordo N (2001) Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. J Virol 75:3268–3276

Barberis I, Myles P, Ault SK, Bragazzi NL, Martini M (2016) History and evolution of influenza control through vaccination: from the first monovalent vaccine to universal vaccines. J Prev Med Hyg 57:E115–E120

Barr T (1889) Two cases of total loss of hearing in both ears, consequent upon mumps, with observations. Glasgow Med J 31:408–412

Bedford T, Malik HS (2016) Did a single amino acid change make Ebola virus more virulent? Cell 167:892–894

Bogdanoff WA, Perez EI, López T, Arias CF, DuBois RM (2017) Structural basis for escape of human astrovirus from antibody neutralization: broad implications for rational vaccine design. J Virol. 92:e01546–e1617

Brown EG, Dimock K, Wright KE (1996) The Urabe AM9 mumps vaccine is a mixture of viruses differing at amino acid 335 of the hemagglutinin-neuraminidase gene with one form associated with disease. J Inf Dis 174:619–622

Burne J (1851) Case of epidemic mumps: complicated with parotitis, orchitis, nephritis, albuminuria, convulsions: recovery. Prov Med Surg J 15:623–625

Buynak EB, Weibel RE, Whitman JE Jr, Stokes J Jr, Hilleman MR (1969) Combined live measles, mumps, and rubella virus vaccines. JAMA 207:2259–2262

Cagno V, Donalisio M, Civra A, Volante M, Veccelli E, Oreste P, Rusnati M, Lembo D (2014) Highly sulfated K5 *Escherichia coli* polysaccharide derivatives inhibit respiratory syncytial virus infectivity in cell lines and human tracheal-bronchial histocultures. Antimicrob Agents Chemother 58:4782–4794

Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, Dal Cin P, Dye JM, Whelan SP, Chandran K, Brummelkamp TR (2011) Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 477:340–343

Carp RI (1963) A study of the mutation rates of several poliovirus strains to the reproductive capacity temperature/40+ and guanidine marker characteristics. Virology 21:373–382

Carpenter JA, Keegan LP, Wilfert L, O'Connell MA, Jiggins FM (2009) Evidence for ADAR-induced hypermutation of the Drosophila sigma virus (Rhabdoviridae). BMC Genet 26(10):75

Carrillo-Tripp M, Shepherd CM, Borelli IA et al (2009) VIPERdb2: an enhanced and web API enabled relational database for structural virology|Nucleic Acids Research|Oxford Academic. Nucleic Acids Res 37:D436–D442

Chang-Jer W, Jia-Tsrong J, Chi-Min C, Hsing-Pang H, Der-Ren H, Hwan-WL C-Y, Hui-Wen H, Su-Chin C, Cheng-Fong H, Ren-Kuo L, Yu-Sheng C, John TAH (2004) Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob Agents Chemother 48:2693–2696

Chattopadhyay S, Sinha NK, Banerjee S, Roy D, Chattopadhyay D, Roy S (2006) Small cationic protein from a marine turtle has β-defensin-like fold and antibacterial and antiviral activity, Proteins Struct Funct Bioinf 64

Chaudhry Y, Nayak A, Bordeleau ME, Tanaka J, Pelletier J, Belsham GJ, Roberts LO, Goodfellow IG (2006) Caliciviruses differ in their functional requirements for eIF4F components. J Biol Chem 281:25315–25325

Chen R, Mukhopadhyay S, Merits A, Bolling B, Nasar F, Coffey LL, Powers A, Weaver SC, Ictv Report Consortium (2018) ICTV virus taxonomy profile: *togaviridae*. J Gen Virol. 99:761–762. https://doi.org/10.1099/jgv.0.001072

Cholleti H, Hayer J, Chanisso Mulandane F, Falk K, Fafetine J, Berg M, Blomström A (2018) Viral metagenomics reveals the presence of highly divergent quaranjavirus in *Rhipicephalus* ticks from Mozambique. Infect Ecol Epidemiol 8:1478585

Chu DK, Poon LL, Guan Y, Peiris JS (2008) Novel astroviruses in insectivorous bats. J Virol 82(18):9107–9114

Chuang JL, Jackson RL, Perrault J (1997) Isolation and characterization of vesicular stomatitis virus PoIR revertants: polymerase readthrough of the leader-N gene junction is linked to an ATP-dependent function. Virology 229:57–67

Chumakov KM, Dragunsky EM, Norwood LP, Douthitt MP, Ran Y, Taffs RE, Ridge J, Levenbook IS (1994) Consistent selection of mutations in the 5'-untranslated region of oral poliovirus vaccine upon passaging in vitro. J Med Virol 42:79–85

Clark HF (1980) Rabies serogroup viruses in neuroblastoma cells: propagation, "autointerference," and apparently random back-mutation of attenuated viruses to the virulent state. Infect Immun 27:1012–1022

Clark HF, Koprowski H (1971) Isolation of temperature-sensitive conditional lethal mutants of "fixed" rabies virus. J Virol 7:295–300

Cleverley DZ, Lenard J (1998) The transmembrane domain in viral fusion: essential role for a conserved glycine residue in vesicular stomatitis virus G protein. Proc Natl Acad Sci U S A 95:3425–3430

Collin EA, Sheng Z, Lang Y, Ma W, Hause BM, Li F (2015) Cocirculation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle. J Virol 89:1036–1042

Combe M, Sanjuán R (2014) Variation in RNA virus mutation rates across host cells. PLoS Pathog 10:e1003855

Conzelmann KK, Cox JH, Schneider LG, Thiel HJ (1990) Molecular cloning and complete nucleotide sequence of the attenuated rabies virus SAD B19. Virology 175:485–499

Corman VM, Muth D, Niemeyer D, Drosten C (2018) Hosts and sources of endemic human coronaviruses. Adv Virus Res 100:163–188

Decroly E, Ferron F, Lescar J, Canard B (2012) Conventional and unconventional mechanisms for capping viral mRNA. Nat Rev Microbiol 10:51–65

de Groot RJ, Baker SC, Baric R, Enjuanes L, Gorbalenya AE, Holmes KV, Perlman S, Poon L, Rottier PJ, Talbot PJ, Woo PC, Ziebuhr J (2011) Family Coronaviridae. In: King AMQ, Lefkowitz E, Adams MJ, Carstens EB (eds) Ninth Report of the International

Committee on Taxonomy of Viruses. Elsevier, Oxford, pp 806–828

Derdeyn CA, Frey TK (1995) Characterization of defective-interfering RNAs of rubella virus generated during serial undiluted passage. Virology 206:216–226

Diehl WE, Lin AE, Grubaugh ND, Carvalho LM, Kim K, Kyawe PP, McCauley SM, Donnard E, Kucukural A, McDonel P, Schaffner SF, Garber M, Rambaut A, Andersen KG, Sabeti PC, Luban J (2016) Ebola virus glycoprotein with increased infectivity dominated the 2013–2016 epidemic. Cell 167:1088–1098

Dietzgen RG, Kondo H, Goodin MM et al (2017) The family Rhabdoviridae: mono- and bipartite negative-sense RNA viruses with diverse genome organization and common evolutionary origins. Virus Res 227:158–170

Dilcher M, Barratt K, Douglas J, Strathdee A, Anderson T, Werno A (2018) Monitoring viral genetic variation as a tool to improve molecular diagnostics for mumps virus. J Clin Microbiol 56(10):e00405–418

Domegan LM, Atkins GJ (2002) Apoptosis induction by the Therien and vaccine RA27/3 strains of rubella virus causes depletion of oligodendrocytes from rat neural cell cultures. J Gen Virol 83:2135–2143

Domingo E, Menéndez-Arias L, Holland JJ (1997) RNA virus fitness. Rev Med Virol 7:87–96

Drake JW (1993) Rates of spontaneous mutation among RNA viruses. Proc Natl Acad Sci USA 90:4171–4175

Duffy S (2018) Why are RNA virus mutation rates so damn high? PLoS Biol 16:e3000003

Dunn G, Begg NT, Cammack N, Minor PD (1990) Virus excretion and mutation by infants following primary vaccination with live oral poliovaccine from two sources. J Med Virol 32:92–95

Durrer P, Gaudin Y, Ruigrok RW, Graf R, Brunner J (1995) Photolabeling identifies a putative fusion domain in the envelope glycoprotein of rabies and vesicular stomatitis viruses. J Biol Chem 270:17575–17581

Dussurget O, Bierne H, Cossart P (2014) The bacterial pathogen Listeria monocytogenes and the interferon family: type I, type II and type III interferons. Front Cell Infect Microbiol 4:50

Easterday BC, Laver WG, Pereira HG, Schild GC (1969) Antigenic composition of recombinant virus strains produced from human and avian influenza. J Gen Virol 5:83–91

Faber M, Faber ML, Li J, Preuss MA, Schnell MJ, Dietzschold B (2007) Dominance of a nonpathogenic glycoprotein gene over a pathogenic glycoprotein gene in rabies virus. Virol 81:7041–7047

Faber M, Faber ML, Papaneri A, Bette M, Weihe E, Dietzschold B, Schnell MJ (2005) A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. J Virol 79:14141–14148

Finke S, Granzow H, Hurst J, Pollin R, Mettenleiter TC (2010) Intergenotypic replacement of lyssavirus matrix proteins demonstrates the role of lyssavirus M proteins in intracellular virus accumulation. J Virol 84:1816–1827

Fitzsimmons WJ, Woods RJ, McCrone JT et al (2018) A speed–fidelity trade-off determines the mutation rate and virulence of an RNA virus. PLoS Biol 16:e2006459. https://doi.org/10.1371/journal.pbio.2006459

Flamand A (1970) Genetic study of vesicular stomatitis virus: classification of spontaneous thermosensitive mutants into complementation groups. J Gen Virol 8:187–195

Fredericksen BL, Whitt MA (1995) Vesicular stomatitis virus glycoprotein mutations that affect membrane fusion activity and abolish virus infectivity. J Virol 69:1435–1443

Furuichi Y, Shatkin AJ (2000) Viral and cellular mRNA capping: past and prospects. Ad Virus Res 55:135

Gasparini R, Amicizia D, Lai PL, Bragazzi NL, Panatto D (2014) Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza. Part I: influenza life-cycle and currently available drugs. J Prev Med Hyg 55:69–85

Gaudin Y, Ruigrok RW, Tuffereau C, Knossow M, Flamand A (1992) Rabies virus glycoprotein is a trimer. Virology 187:627–632

Gholami A, Kassis R, Real E, Delmas O, Guadagnini S, Larrous F, Obach D, Prevost MC, Jacob Y, Bourhy H (2008) Mitochondrial dysfunction in lyssavirus-induced apoptosis. J Virol 82:4774–4784

Gilhespy FB, Holden HS (1917) Grave diabetes mellitus with pulmonary tuberculosis following mumps. Br Med J 2:115

Gitlin L, Karelsky S, Andino R (2002s) Short interfering RNA confers intracellular antiviral immunity in human cells. Nature 418:430–434

Griebel CR, Schnee K (1950) Fatal meningitis following epidemic parotitis. Munch Med Wochenschr 92:593–595

Halfmann P, Ebihara H, Marzi A, Hatta Y, Watanabe S, Suresh M, Neumann G, Feldmann H, Kawaoka Y (2009) Replication-deficient ebolavirus as a vaccine candidate. J Virol 83:3810–3815

Hamilton R (1790) An account of a distemper, by the common people in england vulgarly called the mumps. Lond Med J 11:190–211

Hause BM, Collin EA, Liu R, Huang B, Sheng Z, Lu W, Wang D, Nelson EA, Li F (2014) Characterization of a novel influenza virus in cattle and Swine: proposal for a new genus in the *Orthomyxoviridae* family. mBio 5:e00031–e114

Hellebø A, Vilas U, Falk K, Vlasak R (2004) Infectious salmon anemia virus specifically binds to and hydrolyzes 4-O-acetylated sialic acids. J Virol 78:3055–3062

Heneen WK, Nichols WW, Levan A, Norrby E (1970) Polykaryocytosis and mitosis in a human cell line after treatment with measles virus. Hereditas 64:53–84

Hewlett MJ, Axelrod JH, Antinoro N, Feld R (1982) Isolation and preliminary characterization of temperature-sensitive mutants of poliovirus type 1. J Virol 41:1089–1094

Higgins PG, Phillpotts RJ, Scott GM, Wallace J, Bernhardt LL, Tyrrell DA (1983) Intranasal interferon as protection against experimental respiratory coronavirus infection in volunteers. Antimicrob Agents Chemother 24:713–715

Holland JJ, de la Torre JC, Steinhauer DA (1992) RNA virus populations as quasispecies. Curr Top Microbiol Immunol 176:1–20

Hviid A, Rubin S, Mühlemann K (2008) Mumps. Lancet 371:932–944

Ilkow CS, Weckbecker D, Cho WJ, Meier S, Beatch MD, Goping IS, Herrmann JM, Hobman TC (2010) The rubella virus capsid protein inhibits mitochondrial import. J Virol 84:119–130

Isaacs A, Lindenmann J (1957) Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 147:258–267

Ito H, Minamoto N, Watanabe T, Goto H, Rong LT, Sugiyama M, Kinjo T, Mannen K, Mifune K, Konobe T, Yoshida I, Takamizawa A (1994) A unique mutation of glycoprotein gene of the attenuated RC-HL strain of rabies virus, a seed virus used for production of animal vaccine in Japan. Microbiol Immunol 38:479–482

Ito H, Watanabe S, Sanchez A, Whitt MA, Kawaoka Y (1999) Mutational analysis of the putative fusion domain of Ebola virus glycoprotein. J Virol 73:8907–8912

Izuka N, Kohara M, Hagino-Yamagishi K, Abe S, Komatsu T, Tago K, Arita M, Nomoto A (1989) Construction of less neurovirulent polioviruses by introducing deletions into the 5' noncoding sequence of the genome. J Virol 63:5354–5363

Jablonski SA, Morrow (1995) Mutation of the aspartic acid residues of the GDD sequence motif of poliovirus RNA-dependent RNA polymerase results in enzymes with altered metal ion requirements for activity. J Virol 69:1532–1539

Jacob HW (1900) Notes on a case of acute pancreatitis complicating mumps. Br Med J 1:1532–1533

Jenkins GM, Rambaut A, Pybus OG, Holmes EC (2002) Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. J Mol Evol 54:156–165

Jiang S, He Y, Liu S (2005) SARS vaccine development. Emerg Infect Dis 11:1016–1020

Jin L, Örvell C, Myers R, Rota PA, Nakayama T, Forcic D, Hiebert J, Brown KE (2015) Genomic diversity of mumps virus and global distribution of the 12 genotypes. Rev Med Virol 25:85–101

Johansson S, Niklasson B, Maizel J, Gorbalenya AE, Lindberg AM (2002) Molecular analysis of three Ljungan virus isolates reveals a new, close-to-root lineage of the Picornaviridae with a cluster of two unrelated 2A proteins. J Virol 76:8920–8930

Joki-Korpela P, Hyypiä T (2001) Parechoviruses, a novel group of human picornaviruses. Ann Med 33:466–471

Kakizawa J, Nitta Y, Yamashita T, Ushijima H, Katow S (2001) Mutations of rubella virus vaccine TO-336 strain occurred in the attenuation process of wild progenitor virus. Vaccine 19:2793–2802

Ke Q, Weaver W, Pore A, Gorgoglione B, Wildschutte JH, Xiao P, Shepherd BS, Spear A, Malathi K, Stepien CA, Vakharia VN, Leaman DW (2017) Role of viral hemorrhagic septicemia virus matrix (M) protein in suppressing host transcription. J Virol. 91(19):e00279–e317

Kearney K, Menton J, Morgan JG (2007) Carlow virus, a 2002 GII.4 variant Norovirus strain from Ireland. Virol J. 4:61

Kendal AP, Kiley MP (1973) Characterization of influenza virus neuraminidases: peptide changes associated with antigenic divergence between early and late N2 neuraminidases. J Virol 12:1482–1490

Keng C-T, Zhang A, Shen S, Lip K-M, Fielding BC, Tan THP, Chou C-F, Loh CB, Wang S, Fu J, Yang X, Lim SG, Hong W, Tan Y-J (2005) Amino acids 1055 to 1192 in the S2 region of severe acute respiratory syndrome coronavirus s protein induce neutralizing antibodies: implications for the development of vaccines and antiviral agents. J Virol 79:3289–3296

King AMQ, Brown F, Christian P, Hovi T, Hyypiä T, Knowles NJ, Lemon SM, Minor PD, Palmenberg AC, Skern T, Stanway GP (2000) Virus taxonomy. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Calisher CH, Carsten EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch D, Pringle CR, Wickner RB (eds) Seventh Report of the International Committee for the Taxonomy of Viruses. Academic Press, New York, 657–673

Kitamura N, Semeler B, Rothberg P, Larsen G, Adler C, Dorner A, Emini E, Hanecak R, Lee J, van der Werf S, Anderson C, Wimmer E (1981) Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature (London) 291:547–553

Kondratowicz AS, Lennemann NJ, Sinn PL, Davey RA, Hunt CL, Moller-Tank S, Meyerholz DK, Rennert P, Mullins RF, Brindley M, Sandersfeld LM, Quinn K, Weller M, McCray PB Jr, Chiorini J, Maury W (2011) T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. Proc Natl Acad Sci USA 108:8426–8431

Korboukh VK, Lee CA, Acevedo A et al (2014) RNA virus population diversity, an optimum for maximal fitness and virulence. J Biol Chem 289:29531–29544. https://doi.org/10.1074/jbc.M114.592303

Kuhn JH, Amarasinghe GK, Basler CF et al (2019) ICTV virus taxonomy profile: Filoviridae. J Gen Virol 100:911–912

Langevin C, Tuffereau C (2002) Mutations conferring resistance to neutralization by a soluble form of the neurotrophin receptor (p75NTR) map outside of the known antigenic sites of the rabies virus glycoprotein. J Virol 76:10756–10765

Lauring AS, Acevedo A, Cooper SB, Andino R (2012) Codon usage determines the mutational robustness, evolutionary capacity, and virulence of an RNA virus. Cell Host Microbe 12:623–632

Lázaro-Frías A, Gómez-Medina S, Sánchez-Sampedro L, Ljungberg K, Ustav M, Liljeström P, Muñoz-Fontela C, Esteban M, García-Arriaza J (2018) Distinct immunogenicity and efficacy of poxvirus-based vaccine candidates against ebola virus expressing GP and VP40 proteins. J Virol 92:e00363–e418

Lennemann NJ, Walkner M, Berkebile AR, Patel N, Maury W (2015) The role of conserved N-linked glycans on ebola virus glycoprotein 2. J Infect Dis. 212(2):S204–209

Lenski RE, Travisano M (1994) Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. Proc Natl Acad Sci USA 91:6808–6814

Levenshtam MA, Radyshich NS, Zakharchenko EM, Shroit IG (1969) Chromosomal aberrations in human amniotic cells before the completion of a single growth cycle of measles vaccine virus L-16. Acta Virol 13:401–406

Ling Y, Zhengquan L, Kehu Y, Xiuxia Q, Jian C, Guangwen W, Hong Z, Hongpeng L, Lili Z, Pengfei J, Lirong C, Yan S, Min L, Guoying Z, Jianhe H, Deliang D, Yuchun N, Xuanling S, Wei W, Yang H, Taisheng L, Yuqing L, Mingxiao D, Hongkui D, Xiaojie X (2004) Small molecules blocking the entry of severe acute respiratory syndrome coronavirus into host cells. J Virol 78:11334–11339

Liu P, Shi L, Zhang W, He J, Liu C, Zhao C, Kong SK, Loo JFC, Gu D, Hu L (2017) Prevalence and genetic diversity analysis of human coronaviruses among cross-border children. Virol J 14:230

Liu X, Yang X, Lee CA, Moustafa IM, Smidansky ED, Lum D, Arnold JJ, Cameron CE, Boehr DD (2013) Vaccine-derived mutation in motif D of poliovirus RNA-dependent RNA polymerase lowers nucleotide incorporation fidelity. J Biol Chem 288:32753–32765

Lochridge VP, Hardy ME (2007) A single-amino-acid substitution in the P2 domain of VP1 of murine norovirus is sufficient for escape from antibody neutralization. J Virol 81:12316–12322

Lum SH, Turner A, Guiver M, Bonney D, Martland T, Davies E, Newbould M, Brown J, Morfopoulou S, Breuer J, Wynn R (2016) An emerging opportunistic infection: fatal astrovirus (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient. Transpl Infect Dis 18:960–964

Macintyre G, Curry B, Wong F, Anderson R (1991) Hygromycin B therapy of a murine coronaviral hepatitis. Antimicrob Agents Chemother 35:2125–2127

Madeley CR, Cosgrove BP (1975) 28 nm particles in faeces in infantile gastroenteritis. Lancet 2:451–452

Mandary MB, Masomian M, Poh CL (2019) Impact of RNA virus evolution on quasispecies formation and virulence. Int J Mol Sci 20:4657. https://doi.org/10.3390/ijms20184657

Marathe R, Anandalakshmi R, Smith TH, Pruss GJ, Vance VB (2000) RNA viruses as inducers, suppressors and targets of post-transcriptional gene silencing. Plant Mol Biol 43:295–306

Martínez O, Ndungo E, Tantral L, Miller EH, Leung LW, Chandran K, Basler CF (2013) A mutation in the Ebola virus envelope glycoprotein restricts viral entry in a host species- and cell-type-specific manner. J Virol 87:3324–3334

Masatani T, Ito N, Shimizu K, Ito Y, Nakagawa K, Abe M, Yamaoka S, Sugiyama M (2011) Amino acids at positions 273 and 394 in rabies virus nucleoprotein are important for both evasion of host RIG-I-mediated antiviral response and pathogenicity. Virus Res 155:168–174

Matassov D, Marzi A, Latham T, Xu R, Ota-Setlik A, Feldmann F, Geisbert JB, Mire CE, Hamm S, Nowak B, Egan MA, Geisbert TW, Eldridge JH, Feldmann H, Clarke DK (2015) Vaccination with a highly attenuated recombinant vesicular stomatitis virus vector protects against challenge with a lethal dose of ebola virus. J Infect Dis 2:S443–451

Matsui SM, Kiang D, Ginzton N, Chew T, Geigenmüller-Gnirke U (2001) Molecular biology of astroviruses: selected highlights. Novartis Found Symp 238:219–233

McGavin LH (1913) Torticollis following "Mumps" and associated with Bilateral Cervical Ribs. Proc R Soc Med. 6(Clin Sect):26

Mebatsion T, Konig M, Conzelmann KK (1996) Budding of rabies virus particles in the absence of the spike glycoprotein. Cell 84:941–951

Méndez E, Muñoz-Yañez C, Sánchez-San Martín C, Aguirre-Crespo G, Baños-Lara Mdel R, Gutierrez M, Espinosa R, Acevedo Y, Arias CF, López S (2014) Characterization of human astrovirus cell entry. J Virol 88:2452–2460

Meng T, Kwang J (2014) Attenuation of human enterovirus 71 high-replication-fidelity variants in AG129 mice. J Virol 88:5803–5815

Mina MJ, Kula T, Leng Y, Li M, de Vries RD, Knip M, Siljander H, Rewers M, Choy DF, Wilson MS, Larman HB, Nelson AN, Griffin DE, de Swart RL, Elledge SJ (2019) Measles virus infection diminishes preexisting antibodies that offer protection from other pathogens. Science 366:599–606

Mitra T, Sosnovtsev SV, Green KY (2004) Mutagenesis of tyrosine 24 in the VPg protein is lethal for feline calicivirus. J Virol 78:4931–4935

Mosser AG, Sgro JY, Rueckert RR (1994) Distribution of drug resistance mutations in type 3 poliovirus identifies three regions involved in uncoating functions. J Virol 68:8193–8201

Mpanju OM, Towner JS, Dover JE, Nichol ST, Wilson CA (2006) Identification of two amino acid residues on Ebola virus glycoprotein 1 critical for cell entry. Virus Res 121:205–214

Muñoz-Alía MÁ, Muller CP, Russell SJ (2017) Antigenic drift defines a new D4 subgenotype of measles virus. J Virol. 91(11):e00209–e217

Nakagawa K, Ito N, Masatani T, Abe M, Yamaoka S, Ito Y, Okadera K, Sugiyama M (2012) Generation of a live rabies vaccine strain attenuated by multiple mutations and evaluation of its safety and efficacy. Vaccine 30:3610–3617

Nakagawa K, Kobayashi Y, Ito N, Suzuki Y, Okada K, Makino M, Goto H, Takahashi T, Sugiyama M (2017) Molecular function analysis of rabies virus RNA polymerase L protein by using an L gene-deficient virus. J Virol 91:e00826–917

Negredo A, Palacios G, Vázquez-Morón S, González F, Dopazo H, Molero F, Juste J, Quetglas J, Savji N, de la Cruz MM, Herrera JE, Pizarro M, Hutchison SK, Echevarría JE, Lipkin WI, Tenorio A (2011) Discovery of an ebolavirus-like filovirus in Europe. PLoS Pathog 7:e1002304

Nichols WW (1963) Relationships of viruses, chromosomes and carcinogenesis. Ibid 50:53–80

Nichols WW, Levan A, Aula P, Norrby E (1965) Chromosome damage associated with the measles virus in vitro. Hereditas 54:101–118

Novella IS, Duarte EA, Elena SF, Moya A, Domingo E, Holland JJ (1995) Exponential increases of RNA virus fitness during large population transmissions. Proc Natl Acad Sci USA 92:5841–5844

Novella IS, Gilbertson DL, Borrego B, Domingo E, Holland JJ (2005) Adaptability costs in immune escape variants of vesicular stomatitis virus. Virus Res 107:27–34

Novella IS, Presloid JB, Zhou T, Smith-Tsurkan SD, Ebendick-Corpus BE, Dutta RN, Lust KL, Wilke CO (2010) Genomic evolution of vesicular stomatitis virus strains with differences in adaptability. J Virol 84:4960–4968

Novella IS, Zárate S, Metzgar D, Ebendick-Corpus BE (2004) Positive selection of synonymous mutations in vesicular stomatitis virus. J Mol Biol 342:1415–1421

Olabode AS, Jiang X, Robertson DL, Lovell SC (2015) Ebolavirus is evolving but not changing: no evidence for functional change in EBOV from 1976 to the 2014 outbreak. Virology 482:202–207

Ou W, King H, Delisle J, Shi D, Wilson CA (2010) Phenylalanines at positions 88 and 159 of Ebolavirus envelope glycoprotein differentially impact envelope function. Virology 396:135–142

Parker JC, Klintworth GK, Graham DG (1971) Infections of the human central nervous system by myxovirus-paramyxovirus following the attenuated measles virus vaccine. J Neuropathol Exp Neurol 30:125

Parvin JD, Moscona A, Pan WT, Leider JM, Palese P (1986) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. J Virol 59:377–383

Pereira HG (1969) Antigenic variation in influenza viruses in man and animals. Proc R Soc Med 62:43–44

Pereira HG, Rinaldi A, Nardelli L (1967) Antigenic variation among avian influenza A viruses. Bull World Health Organ 37:553–558

Petrova VN, Sawatsky B, Han AX, Laksono BM, Walz L, Parker E, Pieper K, Anderson CA, de Vries RD, Lanzavecchia A, Kellam P, von Messling V, de Swart RL, Russell CA (2019) Incomplete genetic reconstitution of B cell pools contributes to prolonged immunosuppression after measles. Sci Immunol 4:6125

Potter CW (2001) A history of influenza. J Appl Microbiol 91:572–579

Pringle CR (1970) Genetic characteristics of conditional lethal mutants of vesicular stomatitis virus induced by 5-fluorouracil, 5-azacytidine, and ethyl methane sulfonate. J Virol 5:559–567

Pringle CR (1999) Virus taxonomy at the XIth International Congress of Virology, Sydney, Australia. Adv Virol 144:2065–2070

Prins KC, Delpeut S, Leung DW, Reynard O, Volchkova VA, Reid SP, Ramanan P, Cárdenas WB, Amarasinghe GK, Volchkov VE, Basler CF (2010) Mutations abrogating VP35 interaction with double-stranded RNA render Ebola virus avirulent in guinea pigs. J Virol 84:3004–3015

Pugachev KV, Frey TK (1998) Effects of defined mutations in the 5' nontranslated region of rubella virus genomic RNA on virus viability and macromolecule synthesis. J Virol 72:641–650

Racaniello VR, Meriam C (1986) Poliovirus temperature-sensitive mutant containing a single nucleotide deletion in the 5'-noncoding region of the viral RNA. Virology 155:498–507

Reddy T, Shorthouse D, Parton DL et al (2015) Nothing to sneeze at: a dynamic and integrative computational model of an influenza A virion. Structure 23:584–597. https://doi.org/10.1016/j.str.2014.12.019

Rima B, Balkema-Buschmann A, Dundon WG et al (2019) ICTV virus taxonomy profile: paramyxoviridae. J Gen Virol 100:1593–1594. https://doi.org/10.1099/jgv.0.001328

Rima BK, Earle JAP, Baczko K, ter Meulen V, Liebert UG, Carstens C, Carabana J, Caballero M, Celma ML, FernandezMunoz R (1997) Sequence divergence of measles virus haemagglutinin during natural evolution and adaptation to cell culture. J Gen Virol 78:97–106

Rosenberg DH (1945) Acute myocarditis in mumps (epidemic parotitis). Arch Intern Med (Chic) 76:257–263

Sakata M, Komase K, Nakayama T (2009) Histidine at position 1042 of the p150 region of a KRT live attenuated rubella vaccine strain is responsible for the temperature sensitivity. Vaccine 27:234–242

Sanjuán R, Elena SF (2006) Epistasis correlates to genomic complexity. Proc Natl Acad Sci USA 103:14402–14405

Sanjuán R, Moya A, Elena SF (2004a) The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. Proc Natl Acad Sci USA 101:8396–8401

Sanjuán R, Moya A, Elena SF (2004b) The contribution of epistasis to the architecture of fitness in an RNA virus. Proc Natl Acad Sci USA 101:15376–15379

Sasaki J, Kusuhara Y, Maeno Y, Kobayashi N, Yamashita T, Sakae K, Takeda N, Taniguchi K (2001) Construction of an infectious cDNA clone of Aichi virus (a new member of the family

Picornaviridae) and mutational analysis of a stem-loop structure at the 5' end of the genome. J Virol 75:8021–8030

Sato G, Kobayashi Y, Motizuki N, Hirano S, Itou T, Cunha EM, Ito FH, Sakai T (2009) A unique substitution at position 333 on the glycoprotein of rabies virus street strains isolated from non-hematophagous bats in Brazil. Virus Genes 38:74–79

Schild GN, Newman RW (1969) Immunological relationships between the neuraminidases of human and animal influenza viruses. Bull World Health Organ 41:437–445

Schinkel SCB, Rubin S, Wright KE (2017) Mechanisms of temperature sensitivity of attenuated Urabe mumps virus. Virus Res 227:104–109

Schlegel M, Osterwalder JJ, Galeazzi RL, Vernazza PL (1999) Comparative efficacy of three mumps vaccines during disease outbreak in eastern Switzerland: cohort study. BMJ 319:352

Schnettler E, Sreenu VB, Mottram T, McFarlane M (2016) *Wolbachia* restricts insect-specific flavivirus infection in *Aedes aegypti* cells. J Gen Virol 97:3024–3029

Scholte FEM, Zivcec M, Dzimianski JV, Deaton MK, Spengler JR, Welch SR, Nichol ST, Pegan SD, Spiropoulou CF, Bergeron É (2017) Crimean-Congo hemorrhagic fever virus suppresses innate immune responses via a ubiquitin and ISG15 specific protease. Cell Rep 20:2396–2407

Schott DH, Cureton DK, Whelan SP, Hunter CP (2005) An antiviral role for the RNA interference machinery in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 102:18420–18424

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

Seif I, Coulon P, Rollin PE, Flamand A (1985) Rabies virulence: effect on pathogenicity and sequence characterization of rabies virus mutations affecting antigenic site III of the glycoprotein. J Virol 53:926–934

Seto JT, Rott R (1966) Functional significance of sialidase during influenza virus multiplication. Virology 30:731–737

Sexton NR, Smith EC, Blanc H, Vignuzzi M, Peersen OB, Denison MR (2016) Homology-based identification of a mutation in the coronavirus RNA-dependent RNA polymerase that confers resistance to multiple mutagens. J Virol 90:7415–7428

Shah D, Vidal S, Link MA, Rubin SA, Wright KE (2009) Identification of genetic mutations associated with attenuation and changes in tropisms of Urabe mumps virus. J Med Virol 81:130–138

Shi J, Zheng J, Huang H, Hu Y, Bian J, Xu D, Li F (2011) Measles incidence rate and a phylogenetic study of contemporary genotype H1 measles strains in China: is an improved measles vaccine needed? Virus Genes 43:319–326

Shokralla S, Chernish R, Ghosh HP (1999) Effects of double-site mutations of vesicular stomatitis virus glycoprotein G on membrane fusion activity. Virology 256:119–129

Shokralla S, He Y, Wanas E, Ghosh HP (1998) Mutations in a carboxy-terminal region of vesicular stomatitis virus glycoprotein G that affect membrane fusion activity. Virology 242:39–50

Sola I, Almazan F, Zuniga S, Enjuanes L (2015) Continuous and discontinuous RNA synthesis in coronaviruses. Annu Rev Virol 2:265–288

Sosnovtsev SV, Belliot G, Chang KO, Onwudiwe O, Green KY (2005) Feline calicivirus VP2 is essential for the production of infectious virions. J Virol 79:4012–4024

Sullivan NJ, Geisbert TW, Geisbert JB, Shedlock DJ, Xu L, Lamoreaux L, Custers JH, Popernack PM, Yang ZY, Pau MG, Roederer M, Koup RA, Goudsmit J, Jahrling PB, Nabel GJ (2006) Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. PLoS Med 3:e177

Sun Y, Wang Z, Tao J, Wang Y, Wu A, Yang Z, Wang K, Shi L, Chen Y, Guo D (2014) Yeast-based assays for the high-throughput screening of inhibitors of coronavirus RNA cap guanine-N7-methyltransferase. Antivir Res 104:156–164

Szanto AG, Nadin-Davis SA, White BN (2008) Complete genome sequence of a raccoon rabies virus isolate. Virus Res 136:130–139

Taffs RE, Chumakov KM, Rezapkin GV, Lu Z, Douthitt M, Dragunsky EM, Levenbook IS (1995) Genetic stability and mutant selection in Sabin 2 strain of oral poliovirus vaccine grown under different cell culture conditions. Virology 209:366–373

Takayama-Ito M, Inoue K, Shoji Y, Inoue S, Iijima T, Sakai T, Kurane I, Morimoto K (2006) A highly attenuated rabies virus HEP-Flury strain reverts to virulent by single amino acid substitution to arginine at position 333 in glycoprotein. Virus Res 119:208–215

Talbot PJ, Dionne G, Lacroix M (1988) Vaccination against lethal coronavirus-induced encephalitis with a synthetic decapeptide homologous to a domain in the predicted peplomer stalk. J Virol 62:3032–3036

Tan ELC, Ooi EE, Lin C-Y, Tan HC, Ling AE, Lim B, Stanton LW (2004) Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs. Emerg Infect Dis 10:581–586

Tian D, Luo Z, Zhou M, Li M, Yu L, Wang C, Yuan J, Li F, Tian B, Sui B, Chen H, Fu ZF, Zhao L (2015) Critical role of K1685 and K1829 in the large protein of rabies virus in viral pathogenicity and immune evasion. J Virol 90:232–244

Towner JS, Sealy TK, Khristova ML, Albariño CG, Conlan S, Reeder SA, Quan PL, Lipkin WI, Downing R, Tappero JW, Okware S, Lutwama J, Bakamutumaho B, Kayiwa J, Comer JA, Rollin PE, Ksiazek TG, Nichol ST (2008) Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. PLoS Pathog 4:e1000212

Tuffereau C, Leblois H, Bénéjean J, Coulon P, Lafay F, Flamand A (1989) Arginine or lysine in position 333 of ERA and CVS glycoprotein is necessary for rabies virulence in adult mice. Virology 172:206–212

Tůmová B, Pereira HG (1968) Antigenic relationship between influenza A viruses of human and animal origin. Bull World Health Organ 38:415–420

Twort FW, Twort DN (1921) An investigation of influenza. J Hyg 20:85–98

Desselberger U, Nakajima K, Alfino P, Pedersen FS, Haseltine WA, Hannoun C, Palese P (1978) Biochemical evidence that "new" influenza virus strains in nature may arise by recombination (reassortment). Proc Natl Acad Sci USA 75:3341–3345

Urbanowicz RA, McClure CP, Sakuntabhai A, Sall AA, Kobinger G, Müller MA, Holmes EC, Rey FA, Simon-Loriere E, Ball JK (2016) Human adaptation of ebola virus during the West African outbreak. Cell 167:1079–1087

Veiga E, de Lorenzo V, Fernández LA (2003) Neutralization of enteric coronaviruses with *Escherichia coli* cells expressing single-chain Fv-autotransporter fusions. J Virol 77:13396–13398

Victoria M, Miagostovich MP, Ferreira MS, Vieira CB, Fioretti JM, Leite JP, Colina R, Cristina J (2009) Bayesian coalescent inference reveals high evolutionary rates and expansion of Norovirus populations. Infect Genet Evol 9:927–932

Villa TG, Feijoo-Siota L, Rama JLR, Ageitos JM (2017) Antivirals against animal viruses. Biochem Pharmacol 133:97–116

Villa TG, Feijoo-Siota L, Sánchez-Pérez A (2018) A short voyage into the past: former misconceptions and misinterpretations in the etiology of some viral diseases. Appl Microbiol Biotech 102:7257–7263

Villa-Komaroff L, Guttman N, Baltimore D, Lodishi HF (1975) Complete translation of poliovirus RNA in a eukaryotic cell-free system. Proc Natl Acad Sci USA 72:4157–4561

Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST (2005) Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2:69

Vinjé J, Estes MK, Esteves P et al (2019) ICTV virus taxonomy profile: caliciviridae. J Gen Virol 100:1469–1470

Virojanapirom P, Khawplod P, Sawangvaree A, Wacharapluesadee S, Hemachudha T, Yamada K, Morimoto K, Nishizono A (2012) Molecular analysis of the mutational effects of Thai street rabies virus with increased virulence in mice after passages in the BHK cell line. Arch Virol 157:2201–2205

Volchkov VE, Feldmann H, Volchkova VA, Klenk HD (1998) Processing of the Ebola virus glycoprotein by the proprotein convertase furin. Proc Natl Acad Sci USA 95:5762–5767

Wang B, Yang C, Tekes G, Mueller S, Paul A, Whelan SP, Wimmer E (2015) Recoding of the vesicular stomatitis virus L gene by computer-aided design provides a live, attenuated vaccine candidate. MBio 6:e00237–e315

Watanabe S, Takada A, Watanabe T, Ito H, Kida H, Kawaoka Y (2000) Functional importance of the coiled-coil of the Ebola virus glycoprotein. J Virol 74:10194–10201

Webster RG, Pereira HG (1968) A common surface antigen in influenza viruses from human and avian sources. J Gen Virol 3:201–208

Wheelock EF, Sibley WA (1965) Circulating virus, interferon and antibody after vaccination with the 17-D strain of yellow-fever virus. New Engl J Med 273(4):194–198

Whitt MA, Buonocore L, Prehaud C, Rose JK (1991) Membrane fusion activity, oligomerization, and assembly of the rabies virus glycoprotein. Virology 185:681–688

Wiersma LC, Rimmelzwaan GF, Vries RD (2015) Developing universal influenza vaccines: hitting the nail, not just on the head. Vaccines (Basel) 3:239–262

Wiktor TJ, Gyorgy E, Schlumberger HD, Sokol F, Koprowski H (1973) Antigenic properties of rabies virus components. J Immunol 110:269–276

Willocks LJ, Guerendiain D, Austin HI, Morrison KE, Cameron RL, Templeton KE, De Lima VRF, Ewing R, Donovan W, Pollock KGJ (2017) An outbreak of mumps with genetic strain variation in a highly vaccinated student population in Scotland. Epidemiol Infect 145:3219–3225

Wollstein M (1921) Experimental mumps meningitis. J Exp Med 34:537–540

Xu X, Liu Y, Weiss S, Arnold E, Sarafianos SG, Ding J (2003) Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. Nucleic Acids Res 31:7117–7130

Yamada K, Noguchi K, Nishizono A (2014) Efficient N-glycosylation at position 37, but not at position 146, in the street rabies virus glycoprotein reduces pathogenicity. Virus Res 179:169–176

Yamada K, Park CH, Noguchi K, Kojima D, Kubo T, Komiya N, Matsumoto T, Mitui MT, Ahmed K, Morimoto K, Inoue S, Nishizono A (2012) Serial passage of a street rabies virus in mouse neuroblastoma cells resulted in attenuation: potential role of the additional N-glycosylation of a viral glycoprotein in the reduced pathogenicity of street rabies virus. Virus Res 165:34–45

Yang D, Hwang D, Qiu Z, Gillam S (1998) Effects of mutations in the rubella virus E1 glycoprotein on E1–E2 interaction and membrane fusion activity. J Virol 72:8747–8755

Yao J, Gillam S (2000) A single-amino-acid substitution of a tyrosine residue in the rubella virus E1 cytoplasmic domain blocks virus release. J Virol 74:3029–3036

Young JF, Palese P (1979) Evolution of human influenza A viruses in nature: recombination contributes to genetic variation of H1N1 strains. Proc Natl Acad Sci USA 76:6547–6551

Youngner JS, Wertz G (1968) Interferon production in mice by vesicular stomatitis virus. J Virol 2:1360–1361

Yount B, Curtis KM, Fritz EA, Hensley LE, Jahrling PB, Prentice E, Denison MR, Geisbert TW, Baric RS (2003) Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci USA 100:12995–13000

Yuan S, Cao L, Ling H, Dang M, Sun Y, Zhang X, Chen Y, Zhang L, Su D, Wang X, Rao Z (2015) TIM-1 acts a dual-attachment receptor for Ebolavirus by interacting directly with viral GP and the PS on the viral envelope. Protein Cell 6:814–824

Zell R, Delwart E, Gorbalenya AE, Hovi T, King AMQ, Nowles NJ, Lindberg AM, Pallansch MA, Reuter G, Palmenberg AC, Simmonds P, Skern T, Stanway G, Yamashita T, Ictv Report Consortium (2017) ICTV virus taxonomy profile: picornaviridae. J Gen Virol. 98:2421–2422. https://doi.org/10.1099/jgv.0.000911

Zeng J, Wang H, Xie X, Li C, Zhou G, Yang D, Yu L (2014) Ribavirin-resistant variants of foot-and-mouth disease virus: the effect of restricted quasispecies diversity on viral virulence. J Virol 88:4008–4020

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.