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Historical Discoveries on Viruses in the Environment and Their Impact on Public Health

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Abstract
Background: Transmission of many viruses occurs by direct transmission during a close contact between two hosts, or by an indirect transmission through the environment. Several and often interconnected factors, both abiotic and biotic, determine the persistence of these viruses released in the environment, which can last from a few seconds to several years. Moreover, viruses in the environment are able to travel short to very long distances, especially in the air or in water. ***Summary:*** Although well described now, the role of these environments as intermediaries or as reservoirs in virus transmission has been extensively studied and debated in the last century. The majority of these discoveries, such as the pioneer work on bacteria transmission, the progressive discoveries of viruses, as well as the persistence of the influenza virus in the air varying along with droplet sizes, or the role of water in the transmission of poliovirus, have contributed to the improvement of public health. Recent outbreaks of human coronavirus, influenza virus, and Ebola virus have also demonstrated the contemporaneity of these research studies and the need to study virus persistence in the environment. ***Key Messages:*** In this review, we discuss historical discoveries that contributed to describe biotic and abiotic factors determining viral persistence in the environment.

Introduction

Viruses are the most abundant biological entities on earth with 10^{31} virions [1]. Viral particles are found everywhere, in the wildness and in urban area, on different surfaces, in biological fluids, and in the air, with temperatures ranging from below 0°C to above 30°C [2, 3]. In seawaters, it has been observed that viral particles are present at a higher density compared with bacteria [4, 5]. This great diversity of environment enables the diversification of transmission modes between individuals among a population. Although the transmission of a virus usually depends on the combination of different factors, such as the contact between the virus and a naive host and the replication capacity of this virus in the naive host, the transmission of a virus secreted in the environment also

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relies on its survival outside its host [6, 7]. Abiotic factors such as temperature, humidity, pH, water salinity, or the presence of ultraviolet (UV) light are all driving viral transmission by altering or promoting viral particle persistence in the environment. These abiotic factors can cause a more or less significant bottleneck for virus diversity during transmission, depending on the transmission route, as observed with influenza viruses [8, 9], or on the seasonal variations, as observed with algal viruses [10].

In the past centuries, scientists studying viruses in the environment made major discoveries for the field of infectious diseases. Their results contributed to understanding the sources of infection as well as pathogen transmissions between infected hosts, leading to an evolution of public health policies. This review presents the history of environmental virology, from a period before virus discovery, where the environment was not considered as a vector until our contemporary era, where the use of modern techniques unveiled new questions on the role played by the environment in virus transmission [11].

Early Discoveries on the Role of the Environment as Reservoir

In the ancient times, Persian physicians such as Muhammad ibn Zakariya al-Razi (Rhazes) in the 9th century and Ibn Sina (Avicenna) in the 10th century developed pioneer theories on infectious diseases, in which the origins of diseases such as variola, measles, and tuberculosis are external agents present in the contaminated air or water [12, 13] (Table 1). Later, Sayyid Ismail Jorjani would even recommend boiling or filtering water before consumption [13, 14]. In 1546, three centuries before the germ theory was admitted and the miasma theory was consequently buried, the Italian physician Girolamo Fracastoro wrote the book *De contagione et contagiosis morbis*. In this book, he defined the contagion as the transmission of an invisible agent, which could be direct by contact, indirect by fomites, or to a distance [15, 16]. Although the theory of G. Fracastoro was controversial at this time, his ideas remained influential on the later conception of epidemics and played a role in the response against following tuberculosis outbreaks [17]. However, he assumed that this agent was a chemical substance, rather than a living microorganism [18]. Two centuries later, during Pontiac's war of 1763, the British army contrived to spread smallpox disease on Delaware Indians, using infected blankets [19]. Although this is a famous example in the history of biological warfare, it is not clear if the trial was successful. However, this event illustrates that the possibility of smallpox virus transmission by contact with an infected surface was accepted.

In the 17th century, Antonie van Leeuwenhoek improved the microscopy by creating >300 microscopes and cutting-edge lenses made by himself. Thanks to this technical revolution, the nascent microbiology became a more established science, moving forward to observation and experimentation. The awareness of the environment as a reservoir for microorganisms probably really started with Louis Pasteur in 1861 and his germ theory [20] and the German hygienist Carl Flügge, who demonstrated that droplets containing *Mycobacterium tuberculosis* bacilli, the causative agent of tuberculosis, could remain in the air for several hours [21]. Since then, it is acknowledged that the air is filled with microscopic germs. Even if viruses were not yet discovered and only bacteria were observable, this major discovery allowed developing new techniques for asepsis and sterilization. In the 2nd half of the 19th century, Joseph Lister developed the sterilization of surgery instruments with phenol and Charles Chamberland invented the autoclave.

At the beginning of the 20th century, new infectious agents called filterable agents were discovered based on their ability to pass through an earthenware filter that retains bacteria, also invented by C. Chamberland [22]. The 1st filterable agent discovered was the tobacco mosaic virus by D. Iwanowski, although M.W. Beijerinck was the 1st to propose that this agent was a virus 6 years later, in 1898 [23]. The same year, Loeffler and Frosch [24] discovered the foot-and-mouth disease virus. During the following 20 years, more than 40 viruses were discovered, including the yellow fever virus, rabies virus, dengue virus, variola virus, poliovirus, and measles virus [25]. In 1918, Charles Nicolle and René Dujarric de la Rivière both simultaneously demonstrated the filterable nature of the agent of influenza, although their results were largely unnoticed [26, 27]. The influenza virus was eventually isolated again 13 years later [28]. During this period of new discoveries, one-third of all deaths registered in the USA were due to 3 leading causes: pneumonia, tuberculosis, and diarrhoea/enteritis [29, 30]. In addition, the pandemic influenza A virus of 1918 was responsible for the highest annual mortality rates of the 20th century, causing at least 40 million deaths worldwide [31]. Except for tuberculosis, whose etiologic agent is a bacterium, respiratory and enteric viruses were thus responsible for a large number of deaths. The transmission modes of these viruses involve a step outside their host, and necessarily their spreading efficiency is dependent on their ability to

Table 1. Early discoveries on pathogen transmission and persistence in the environment

Period	Discovery	Pathogen/disease	Authors	Ref
9–11th centuries	Infection is caused by an external agent present in contaminated air/water	Measles, variola, and tuberculosis	Muhammad ibn Zakariya al-Razi; ibn Sina; Sayyid Ismail Jorjani	[12–14]
1546	Pathogens are invisible and transmitted by direct contact or indirect by fomites	Measles, plague, and typhus	Girolamo Fracastoro	[15, 16]
17th century	Improvement of the microscopes	–	Antonie van Leeuwenhoek	–
1861	The germ theory	–	Louis Pasteur	[20]
1897	*M. tuberculosis* remains in the air for several hours within droplets	*M. tuberculosis*	Carl Flügge	[21]
19th century	Sterilization of surgery instruments with phenol	–	Joseph Lister	–
1884	Invention of the autoclave	–	Charles Chamberland	[22]
1892–1898	Discovery of viruses	Tobacco mosaic virus and foot-and-mouth disease virus	D. Iwanowski; M.W. Beijerinck; F. Loeffler; P. Frosch	[23, 24]
1930	Provided experimental evidence of airborne virus transmission	Influenza virus	William Firth Wells	[36, 37]
1943	Demonstrated that the humidity regulates virus persistence in aerosols	Influenza virus	C.G. Loosli et al.	[45]
1943	Evidence of waterborne virus transmission	Poliovirus and hepatitis A virus	J. Rhodes et al.; S.G. Lensen et al.; J.R. Paul et al.; J.R. Neefe et al.	[55, 61–63]
1979	Water as natural medium for the spread of virus among wild birds	Influenza virus	Virginia S. Hinshaw, Robert G. Webster, and Bruce Turner	[78]

persist in the environment. Unfortunately, the lack of knowledge at the beginning of the century regarding the transmission of these viruses prevented an efficient response from the public health services, contributing to their high prevalence rate.

During this period, scientists gradually found evidence of the influence of environmental abiotic factors on virus survival. In 1900, F.W. Elgin [32] demonstrated that the vaccine virus could be preserved at low temperature and wrote, “It has always been the popular assumption that germ life was incompatible with extreme cold and that in fact most germs […] were destroyed by a freezing temperature.” In this article, he concluded, “Points are unreliable when stored for any length of time at any temperature” and “Hot and especially variable temperatures speedily injure vaccine.” It opened the door to many studies describing virus survival in a wide array of different environments. For example, in 1915, after discovering an immune substance against the poliovirus (the serum treatment) in experimentally infected monkeys [33], Simon Flexner assessed the poliovirus survival contained in a central nervous organ from rhesus macaque in anaerobic condition at 37°C [34]. In this study, he inoculated monkeys by intracerebral injection of brain fragments at different time points and concluded that the survival period of the virus in these conditions was between 20 and 30 days.

Despite the evident progression made at this time in the public health area, the full role of the environment in the transmission of viruses was still underestimated. A complete overview of the knowledge during this period comes from Charles V. Chapin, now considered as a pioneer in public health research. In 1910, he published a book to estimate the relative importance of different factors involved in the transmission of infectious diseases [35]. C.V. Chapin wrote in introduction, “We know now that direct contact with the sick, or with healthy carriers of disease germs, is an exceedingly frequent mode of transmission and that infection by means of the air, or from infected articles, is not nearly as common as was formerly believed.” According to him, the transmission mode of pathogens by direct contact is “the most obvious,” whereas the transmissions by fomites, air, food, and drink are very much less important. Nonetheless, his descriptions of the transmission modes mostly reflected the scientific knowledge at this period, mainly focussed on bacteria. Although his writings have been important in the public health history, the scientific concepts in this book have been challenged in the next decades, as more viruses were progressively discovered.

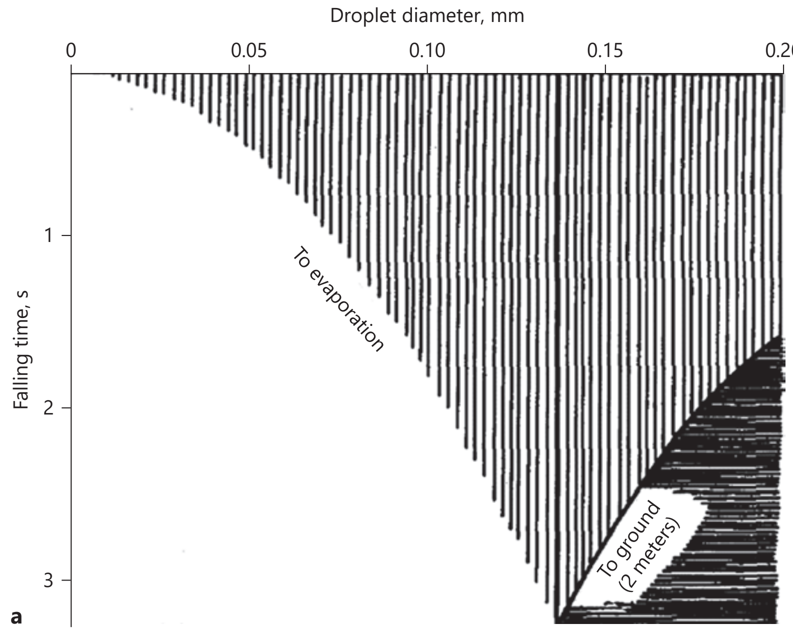
History of Research on Environmental Transmissions: From Basic Knowledge to Public Health Application

Environmental Transmission through the Air

To the best of our knowledge, the oldest experimentation published on airborne transmission of viruses came from the 1930s, with the work of the American scientist William Firth Wells [36]. He established that bioaerosols created by sneezing or coughing carry both viruses and bacteria. Moreover, he developed an air centrifuge [37] to demonstrate that the droplet falling velocity depends on droplet mass, which tends to decrease over the distance due to the evaporation. Indeed, the smallest droplets evaporate faster than they fall and can stay in the air for hours or days (Fig. 1a). These observations have led him to make a distinction between droplets and droplet nuclei, and he postulated that different airborne transmission routes exist depending on the droplet size. This theory is now widely accepted. Wells and Brown [38] performed their 1st demonstrations with bacteria, but soon moved to experiments with influenza virus in 1936, 5 years after the confirmation that the etiologic agent of influenza was a virus [28, 39]. Wells atomized a liquid suspension of influenza virus into a closed tank under irradiation or not with UV light. He then removed a known volume of air at different intervals and collected the suspended material from the air tank to the Wells air centrifuge that he developed himself. He then inoculated ferrets to assess the presence of viable influenza viruses in the collected materials. In conclusion, he observed that ferrets became sick when inoculated with influenza virus previously suspended in the air for 30 min in absence of UV light. On the other hand, ferrets were not sick when viral particles in the air had a prior exposure to UV irradiation. The bactericidal effect of UV was already known at that time, but this experiment was the 1st demonstration that UV also had a virucidal effect.

After this finding, Wells et al. [40] launched in 1937 a 5-year epidemiological trial in different schools, with the purpose of studying the effect of air disinfection with UV lights on the prevalence of mumps, chickenpox, and measles among pupils. UV lights were installed in classrooms, as well as in the music room, the library, the nature room, the hall, the lunchroom, the rest room, and the gymnasium, which were thus all irradiated (Fig. 1b). As a control, several classrooms were kept non-irradiated. Measles, chickenpox, and mumps cases were also recorded among school children for 5 or 9 years prior to the UV light installation in the different schools. Wells et al. [40] noticed an important decrease in mumps, chickenpox, and measles cases during the trial, with no epidemic spread among pupils from the irradiated classrooms, whereas epidemic spread occurred among pupils from the non-irradiated classrooms. As a most significant result, he observed that during the year 1941, 14.5, 15.7, and 9% of susceptible pupils from different schools were infected with measles in irradiated rooms, whereas 55.3 and 51.8% of cases among pupils were recorded in non-irradiated rooms. He concluded that "epidemic contagion is spread through the medium of confined atmospheres and can be prevented by radiant disinfection of air." After that, similar epidemiological studies were also performed in hospitals, with similar results obtained [41]. Similarly, a recent study performed in an elementary school in the USA reported that influenza virus genome was detectable in aerosols (10^1–10^4 genome copies/m^3 air) sampled at different locations during the influenza season [42], suggesting that the air is an important vector for virus transmission in schools.

The environmental persistence of viruses has always been studied both in the field and in the laboratory, allowing us to study the environment as a whole or each variable individually. In order to study experimental airborne infections, different systems were developed. In 1940, Wells [43] published a system composed of an atomizer, a chamber where animals were exposed to infected air, and an incinerator providing airflow in addition to the compressed air bottle and incinerating the effluent air from the animal chamber. In 1943, an English scientist, Derrick G.F. Edward et al. [44], argued that "the use of UV radiation for disinfecting air appears likely in future to become of considerable practical importance." His purpose was to complete the previous epidemiological studies on virus inactivation in the presence of UV with more quantitative results obtained in a laboratory. He designed a set up in which he atomized viral suspension of influenza virus, herpes virus, or vaccinia virus into a cylinder irradiated by using a UV lamp. An irradiation time of 6 s for influenza virus particles and 1 s for herpes virus and vaccinia virus particles was necessary for inactivating these viruses and decontaminating the air. Gradually, lab-



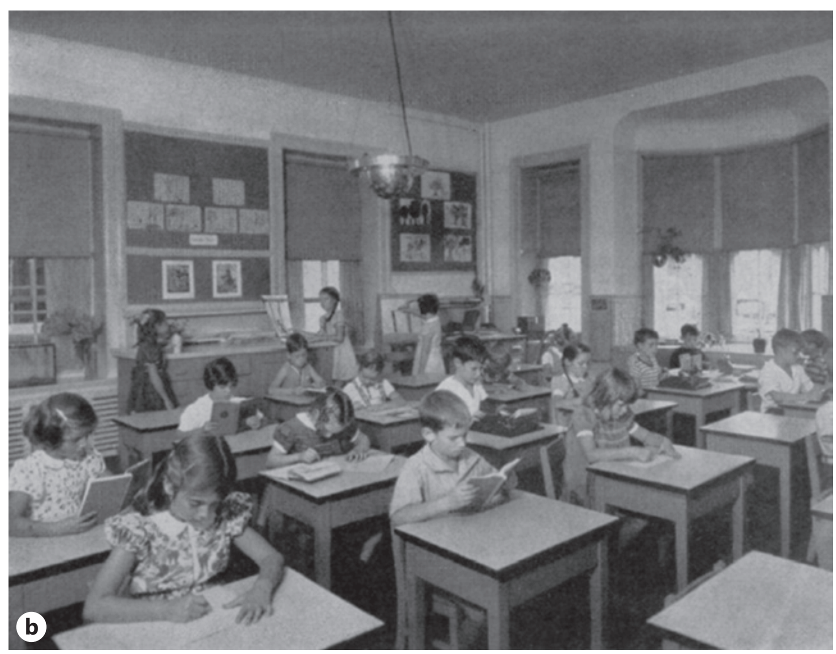


Fig. 1. Early studies on virus persistence in the air. **a** In 1934, Wells [36] published this figure, presenting his results from an experiment assessing the time required for liquid droplets varying in size to fall on the ground, or to evaporate, after being released 2 m above the ground. According to his results, which were obtained in a saturated air, liquid droplets having a diameter a little smaller than 0.15 mm will evaporate before reaching the ground. He also deduced from this data that in the same atmosphere, a droplet of a diameter of 1–10 μm could stay in the air between 10 min and 16 h. Later, W.F. Wells carried on this work by studying the persistence of virus contained in small droplets and deduced that aerosols are a major vector of airborne pathogens. **b** In 1942, Wells et al. [40] published the results of an epidemiological survey in schools, where he tested the effect of the presence of ultraviolet radiation on the epidemics of varicella, measles, and mumps. This photography of a classroom from the Germantown Friends School is presented in his study.

oratory technologies became more complex and research started to focus on the climatic factors driving airborne infection. To this regard, studies performed on the influenza virus were pioneered. In 1943, using a room of 800 cubic feet capacity (23 cubic metres) with controlled temperature and humidity, Loosli et al. [45] demonstrated that influenza virus particles persisted longer in aerosols when the atmosphere had a low level of relative humidity. In their experiments, they sprayed a virus suspension in an atmosphere with a high relative humidity (80–90%) at 27–29°C and observed that the aerosols were no longer infective 1 h after spraying, by placing mice in the chamber to test whether they became infected. In an atmosphere with 45–55% relative humidity, aerosolized par-

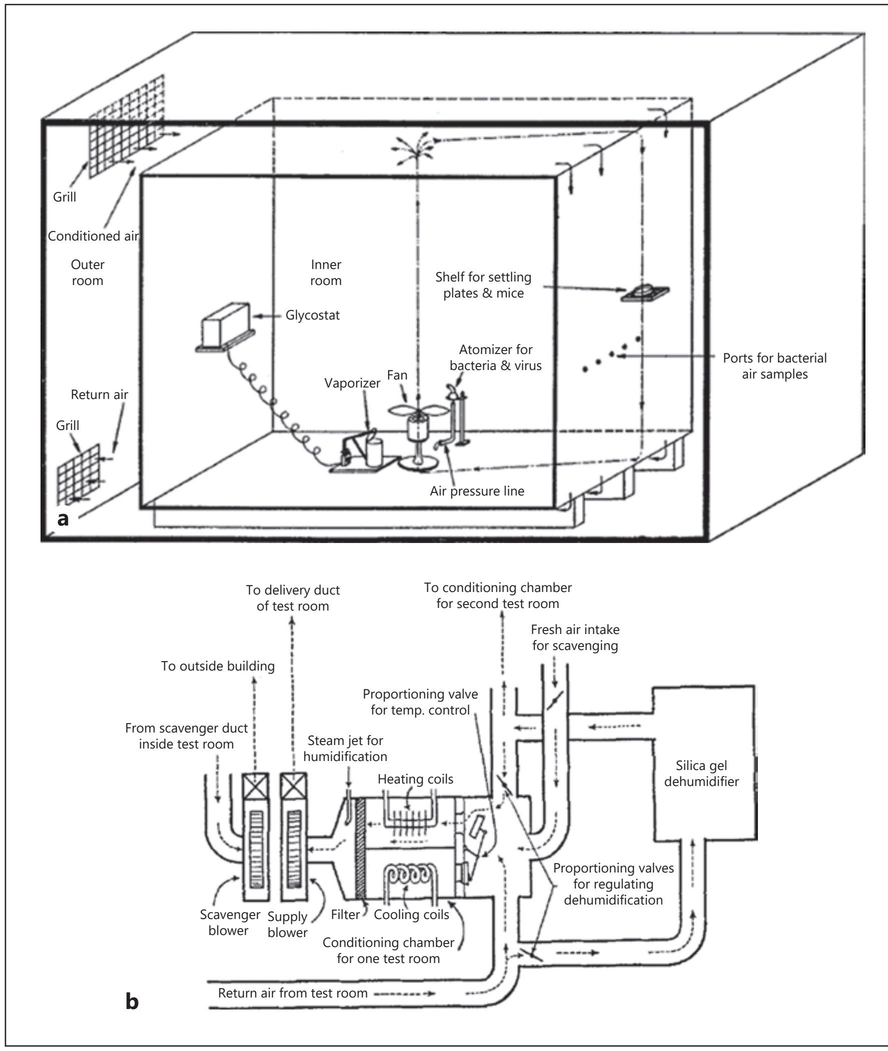


Fig. 2. Experimental chamber for the study of airborne infection. **a** In 1946, Robertson et al. [46] developed a chamber for studying the impact of temperature and humidity on the aerosol transmission of viruses and bacteria. An atomizer was placed in the chamber to spray a bacterial or viral suspension, with an airflow generated by a fan. **b** The schematic plan shows the atmospheric control system, which could control the relative humidity from 12 to 95% and the temperature from 10 to 37°C in the chamber. The relative humidity was increased by the production of steam, and the air temperature was controlled by orienting the airflow in either a heating chamber or a cooling chamber before being released in the main chamber.

ticles remained infective for 6 h. Finally, in a low-humidity (17–24%) atmosphere, viral particles remained infectious for at least 24 h. In 1946, Robertson et al. [46] described the construction of 2 similar chambers of 640 cubic feet capacity for experimental airborne infections (Fig. 2b). By controlling the atmosphere in the room, they observed that the infectivity of influenza virus in the air decreased around 50% relative humidity at 22–23°C, but was kept maximal below 40% relative humidity and above 70% [47], which was partially contradictory with the study by Loosli et al. [45]. In this study, they were also able to increase the virus persistence in a 50% relative humidity atmosphere, by dialyzing the viral suspension prior to its aerosolization in order to separate the salts from the viral particles. They concluded, "The deleterious influence of humidity was related to the presence of sodium chloride in the atomized suspension." Similar results on the effect of humidity were obtained 60 years later with guinea pigs instead of mice [7]. In this more recent work using guinea pigs, Lowen et al. [7] studied the effect of temperature along with relative humidity on influenza virus persistence in the air and concluded that cold and dry conditions favour transmission. In 1968, Mitchell et al. [48] observed that among 14 strains of influenza A viruses, the 6 stains of "human origin were more susceptible to decay than the 8 strains of avian origin" when aerosolized. For this study, the authors used a rotating drum [49], designed to allow a longer persistence of aerosols in suspension, in which temperature and humidity were controlled. Simultaneously, studies were performed to describe the persistence of influenza viruses on various surfaces [50]. Edward et al. [44] reported an uncommon experiment in 1941, where they shook a virus-impregnated blanket and then successfully isolated infectious virus particles distributed in the air after shaking. They also analyzed the virus infectivity after drying on serge, sheet, dust, and glass slides under different temperatures and found that virus particles persisted more on the glass slides. More recently, it has been confirmed that the porosity of a surface negatively affects the persistence of a virus [51, 52].

Environmental Transmission through Water

The poliovirus, which is the causative agent of poliomyelitis, was 1st isolated from the human faeces in 1912 by Swedish scientists [53, 54]. However, in the beginning of the 1940s, poliomyelitis had never been associated with poor drinking water supplies and this virus had not yet been isolated from contaminated waters [55]. In 1943, Maxcy et al. [56] concluded that from the epidemiological point of view, poliomyelitis does not behave like a waterborne disease. They argued that no outbreaks of widely scattered cases were observed in cities with municipal water supplies and that cities with water sources located in remote spots far from human habitation suffered from poliomyelitis as frequently compared with cities that obtained their water from polluted streams [56–58]. Despite different studies showing that the poliovirus was very resistant and remained viable for weeks in stools and water [58–60], the role of water in virus transmission was ignored until the 1940s. This can also be explained partly because the poliovirus was considered as a neurotropic virus only, based on the symptoms caused among patients. Following the development of virus concentration methods, a higher sensitivity of virus detection methods, and more studies performed on the poliovirus survival in water [55, 61] and in sewages [60, 62], as well as the discovery of other waterborne viruses, such as the hepatitis A virus [63], the poliovirus was finally recognized as a waterborne virus. Moreover, Sabin and Ward [64] detected the poliovirus in the human digestive tract in 1941 and demonstrated that it is the primary entry site for poliovirus infection.

Simultaneously, abiotic factors driving virus particles' stability in water were identified, such as the water salinity on influenza virus persistence [65] or the level of oxygen in water [66] that could have a negative impact on virus particles by increasing the oxidation of the viral capsid. Environment-oriented studies were also performed on the hepatitis A virus, in order to develop new disinfection methods of drinking water by comparing the effect of water filtration and the use of different chlorine concentrations [67]. From this period, discoveries in environmental virology and applications in health policy increased over the years. In 1983, Gerald Berg wrote in the introduction of his book, *Viral Pollution of the Environment*, "20 years ago, there were no data showing that there were any viruses present in London's river water, whereas it is now known that every surface water in the Thames Water Authority region which has been examined for viruses has provided a positive result in routine test. To move from a state of total ignorance of contamination to the acceptance of a virtually 100% incidence in less than two decades can have few precedents, and we are faced with a growing list of problems in consequence" [68]. In the 1980s, there was a raising awareness of the virus spread through polluted waters among scientists and public health authorities. Water recreational activities, shellfish harvesting and consumption, drinking water supply, crop irrigation, and aerosolization became diverse sources for a potential virus outbreak [68, 69].

Consequently, several environmental parameters were studied to better understand the persistence of enteroviruses or rotaviruses in water such as the effect of solar radiation, pH, inorganic ions, temperature, water origin (estuarine or marine), and the presence of sludge or biological factors such as protective bacteria [70–72]. All these studies showed that virus survival in the environment was dependent on multiple parameters that are interconnected (Fig. 3). Overall, the temperature was considered as the most important parameter for predicting rhinovirus and poliovirus persistence in water [73, 74]. Moreover, it was observed that rhinovirus, influenza virus, or Newcastle disease virus can lose their infectivity after heating treatment without loss of immunogenicity [75, 76]. In the case of picornaviruses, viral proteins were more rapidly impaired than the viral genome at high temperature, whereas viral genome alteration occurred more rapidly at low temperature [73, 77] (Fig. 3 [78–82]).

Altogether, research studies performed during this period allowed to realize that waterborne viruses can be spread over a long distance in water without a significant loss of infection. In 1979, Hinshaw et al. [83] argued that wild ducks could be the natural reservoir of influenza virus and that water may be a natural medium for the spread of virus among wild birds. To prove it, they isolated infectious virus particles from non-concentrated waters sampled in Alberta lakes. They observed that virus particles remained infectious for 4 days at 22°C and for >30 days at 0°C. During the same decade, it was also observed that waterborne viruses were more stable in aquatic medium when associated with solid particles [84–87].

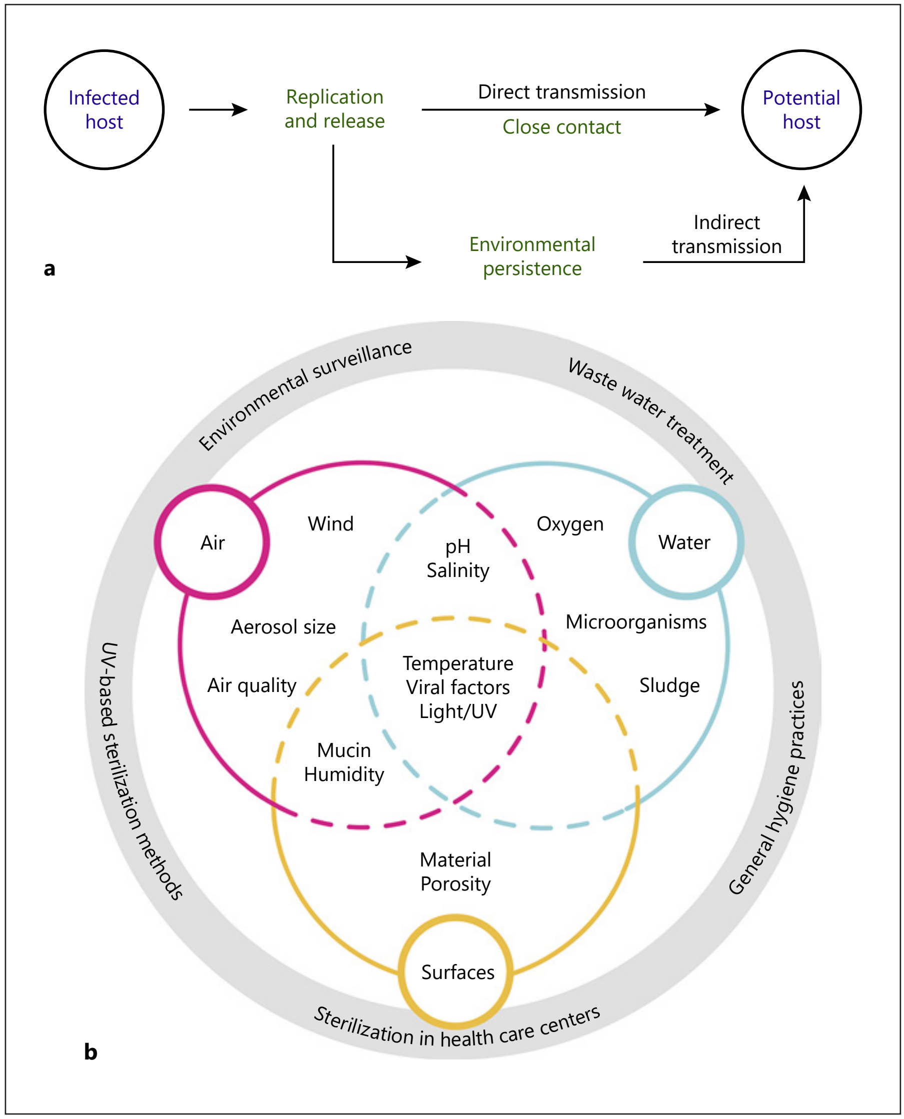


Fig. 3. Multiple factors affecting viral persistence in the environment. **a** After being efficiently replicated and released from an infected host, viruses such as airborne or waterborne viruses can be transmitted to a new naïve host by direct transmission through a close contact, or released in the environment, before encountering a new host. **b** Several factors then determine the persistence of this virus in the environment before a complete loss of infectivity. Among them, the temperature plays a central role in driving environmental persistence. In the air, the temperature affects the relative humidity, which will then modulate the evaporation and thus the size of aerosols. When human respiratory aerosols evaporate, their salinity and pH vary. The mucins enriched in sialic acid and present in these aerosols have a protective role for influenza virus particles [78, 79]. The presence of sludge in water may also provide a protective effect, with the clay adsorbing polio virus particles and protecting them against ultraviolet radiations [70], although it has a negative effect on the rotavirus resistance to heat treatments [71]. In addition to environmental factors, viral factors are also determinants for virion stability in the environment, such as the presence of mutations in the structural proteins [80, 81], or the lipid composition for enveloped viruses [82]. The study of these factors affecting virus persistence contributed greatly to the development of virus control methods and public health in the population, which are summarized in the grey circle.

The field of environmental virology allowed to improve water sanitation and public health policies [57, 69], leading to more rigorous standards and laws in the USA related to safe drinking water [69, 88]. Different disinfection methods such as ozonation [89] and chlorine dioxide [90] were developed as alternative to chlorination used for many years [61, 91, 92] but responsible for the production of suspected carcinogenic compounds in water and used in higher concentration than necessary to be effective. Ozone and chlorine dioxide were already used in Europe at that time [68], and ozone is still a reference for drinking water production. In addition, new antiviral factors, still not completely defined nowadays, have been found in marine water [93–95]. These factors appeared to be thermolabile and sometimes not filterable, guiding suspicions on algae and bacteria [96]. The bacteria *Vibrio marinus* was later identified as one of these factors. Indeed, it was shown that a lytic suspension of this bacteria added in water was able to inactivate RNA and DNA viruses (poliovirus, adenovirus, influenza virus, echovirus, and *Escherichia coli* phage T1) at a similar rate than the raw sea water naturally carrying this bacteria [93, 97, 98]. Since then, much research has been performed to describe the mechanisms of viral persistence in the environment. Comprehensive reviews on the latest results on virus persistence and detection methodology have been recently published [74, 99].

Viruses in the Environment: Evolution of Concepts

Concepts in the history of environmental virology evolved as more viruses were discovered and as new technologies and detection methods were developed, such as the improvement of microscopes, centrifugation, and other virus concentration procedures. Epidemiological field studies and laboratory research works led to a considerable improvement in disease surveillance and sanitation during the 20th century. In 1980, Bitton [88] wrote in the preface of his book, *Introduction to Environmental Virology*, "Environmental virology is now a discipline in its own right." He claimed, "The last decades have witnessed a significant change in outlook and methodology in the field of environmental virology." Nowadays, studying viruses in the environment remained of importance to describe the ecology and transmission of viruses [74]. The recent development of metagenomic approaches brought an unprecedented advantage, by allowing description of viral communities, the virome, in different ecosystems without preconceived knowledge on their composition. Such approach also brings new challenges, due to the amount of viral sequences obtained from the environment, compared with the limited information they provide regarding biological aspects, such as the host or the transmission modes of these viruses [100]. Nevertheless, viral metagenomic studies have been successfully performed on the air [101–103], freshwater and marine waters [104–107], irrigation water [108], in the desert [109], or on surfaces of the Boston and Barcelona subways [110, 111]. These approaches notably raised new questions on virus carriage by the environment. For example, a marine virus was found in the Namib Desert, leading to the assumption of a virus transportation through the seasonal fog to this area [109]. In-depth analyses of environmental abiotic factors driving the local diversity of viral population, performed in the soil [112] or in waters [113] in different oceans [107], illustrated how environmental factors affect host diversity and thus the viral diversity. For example, a recent ecological and computational study on the influenza virus suggested that variations of environmental persistence between different virus subtypes drive their transmission and thus the viral genetic diversity among aquatic birds [114]. Recently, several works on virus egress from infected cells have described a new mode of virus release, in which multiple viral particles are contained in extracellular vesicles [115], derived either from multivesicular bodies or secretory autophagosomes. This mode of transmission, which allows transmission of several particles in individual cells, has been observed for several non-enveloped viruses with an environmental transmission such as poliovirus [116], rotaviruses, noroviruses [117], and hepatitis A and hepatitis E viruses [118]. It was also demonstrated that these vesicles are very stable in a low-pH environment, as well as in urine, blood, and stools, and thus it is likely that these vesicles increase virus persistence in the environment compared to naked viruses. Moreover, a recent study from Leblanc et al. [119] showed that these enteric viruses have different viral decay patterns on surfaces, in water, and on fruits, with noroviruses presenting a lower stability than the rotaviruses and hepatitis A viruses in these media, raising questions on the molecular determinants explaining these differences.

In addition to the studies of virus in the environment, there is still a significant work focussing on disinfection methods. In particular, a 2-year survey showed that enteroviruses, reoviruses, and adenoviruses remained infectious after a UV treatment in wastewater treatment plants [120]. New UV-based methods were also recently proposed, such as the use of a far-UVC light, which is effective on influenza aerosols without the carcinogenic effects of traditional UV lights on the human skin [121]. Similarly, Nishisaka-Nonaka et al. [122] suggested that UV inactivates the influenza virus by preventing the viral replication without altering the function of the external viral proteins. Interestingly, our previous results showed that after virus inactivation in an aquatic environment, the virus remained able to attach to its cellular receptor, but cannot enter into the cell [80], and presented an unaltered genome [82, 123]. A new visible-light-induced photocatalyst has also been reported for its virucidal effect on influenza virus, allowing degradation of 99% of viral particles in <30 min with a very weak light source [124]. Another approach has been proposed recently for decontaminating surfaces, such as the use of potassium persulfate, which was effective on Newcastle disease virus and influenza virus [125].

Importance of Virus Persistence Studies in the Light of Recent Epidemics

Research on virus stability in the environment has also been propelled to the forefront in investigation after recent major outbreaks. In 2003, a non-identified respiratory virus, the severe acute respiratory syndrome-related coronavirus (SARS coronavirus), caused a worldwide outbreak. During the epidemic, numerous transmission events occurred in hospitals, eventually leading to quarantine of the staff and patients in one of these hospitals. Various investigations tried to unveil the transmission of this virus and the potential role played by the environment. Experimental research measured the stability of 2 animal coronaviruses (infecting mice and swine), as a surrogate for the SARS coronavirus, on hard non-porous surfaces [126]. They observed that viruses remained infectious for days at ambient temperature in a wide range of relative humidity (20–60%) and that at 40°C, a low relative humidity (20%) favoured a longer persistence on surfaces (>120 h) compared with a high atmospheric humidity. Another study was later performed directly with the SARS coronavirus and gave similar results [127]. However, in a study using mathematical models to question the possibility of long-range airborne transmission during the largest hospital outbreak in Hong Kong, the authors concluded that fomites alone are less likely to explain the transmission chain and that long-range airborne transmission was likely involved [128]. Nine years after the SARS coronavirus, the Middle East respiratory syndrome coronavirus (MERS coronavirus) emerged, causing major outbreaks in Middle Eastern countries, with >2,400 cases in the world to date. Because of the wide range of climates in which this virus has been detected, its stability in aerosol was recently explored in different atmospheres [129]. At 25°C and 79% relative humidity, 63% of the MERS coronavirus remained infectious 1 h after its aerosolization, whereas at 38°C and 24% relative humidity, reproducing outdoor conditions from the middle-eastern region, only 4.7% of the virus particles remained infectious after their aerosolization. Very recently, the stability of SARS-CoV-2, the causative agent of the COVID-19 pandemic, was assessed on different surfaces and in aerosols [130]. In this study, the authors report that the SARS-CoV-2 was as persistent as the 2003 SARS coronavirus in aerosols and on surfaces, but more stable on cardboard. In a recent meta-analysis, Kampf et al. [131] reviewed the available information on coronavirus persistence on surfaces and efficient disinfection measures, such as 70% ethanol and hydrogen peroxide. Similar to the influenza virus, a debate exists on the transmission modes of this virus, with strong arguments in favour of a transmission by expelled droplets and aerosols [132]. In 2013, an unprecedented Ebola virus outbreak started in West African countries. Before this outbreak, it was already known that the Ebola virus could remain stable for several weeks on surfaces and >1 h in aerosols under diverse conditions of temperature and humidity [133, 134]. The transmission of the Ebola virus among humans occurs primarily through direct contact with contaminated fluids [135]. Because of this, a sampling investigation was carried out on various surfaces from 3 Ebola treatment centres in Sierra Leone in order to evaluate the efficiency of the decontamination protocols used by healthcare workers [136]. Because of safety conditions, the presence of the virus was only determined by detecting the viral genome. Positive samples were mainly obtained in the immediate environment of the patients, showing that the decontamination protocols used were effective. A high number of transmission events during this epidemic occurred through close contacts with human corpses during mourning and funeral practices. To assess the infectivity over time of corpse-associated virus, macaques were used in laboratory experiments as a surrogate for human bodies. The authors observed that the Ebola virus remained infectious >7 days on the surface of bodies [137].

Despite the research on the influenza particles in aerosols started >80 years ago, the relative importance of the different transmission modes of influenza virus among humans, either airborne or by direct and indirect contacts, is still debated [3, 138], especially in the case of tropical countries where transmission by fomites may dominate [139]. Recently, progress was made on the design of experimental chambers for studying airborne transmission, with animal cages separated by a particle separator module preventing large droplet transmission between the animals [140]. Similarly, Zhou et al. [141] studied the influence of the diameter of influenza virus-laden aerosols exhaled from infected ferrets into the air on virus transmission. They observed that the influenza virus was mainly transmitted through aerosols having a diameter higher than 1.5 μm and suggested for the 1st time that size variations of virus-laden aerosols could exist between different virus strains. The climate is also a factor shaping the physicochemical characteristics of evaporating droplets carrying influenza virus. In fact, when relative humidity decreases, the droplet morphology changes, a phase separation is induced by the loss of water, and the pH decreases while the droplet salinity increases [142]. Another study, using the bacteriophage Phi 6 as a surrogate for enveloped virus, evaluated how the relative humidity, the absolute humidity, and the temperature affect the virus in droplets [143]. This study showed that the Phi 6 virus survived longer at high (above 85%) and low (below 60%) relative humidity, leading to the conclusion that this factor was the most important parameter for predicting virus survival. Noticeably, the relative humidity is function of both the temperature and the absolute humidity. The relative humidity in the air is the ratio of partial pres-

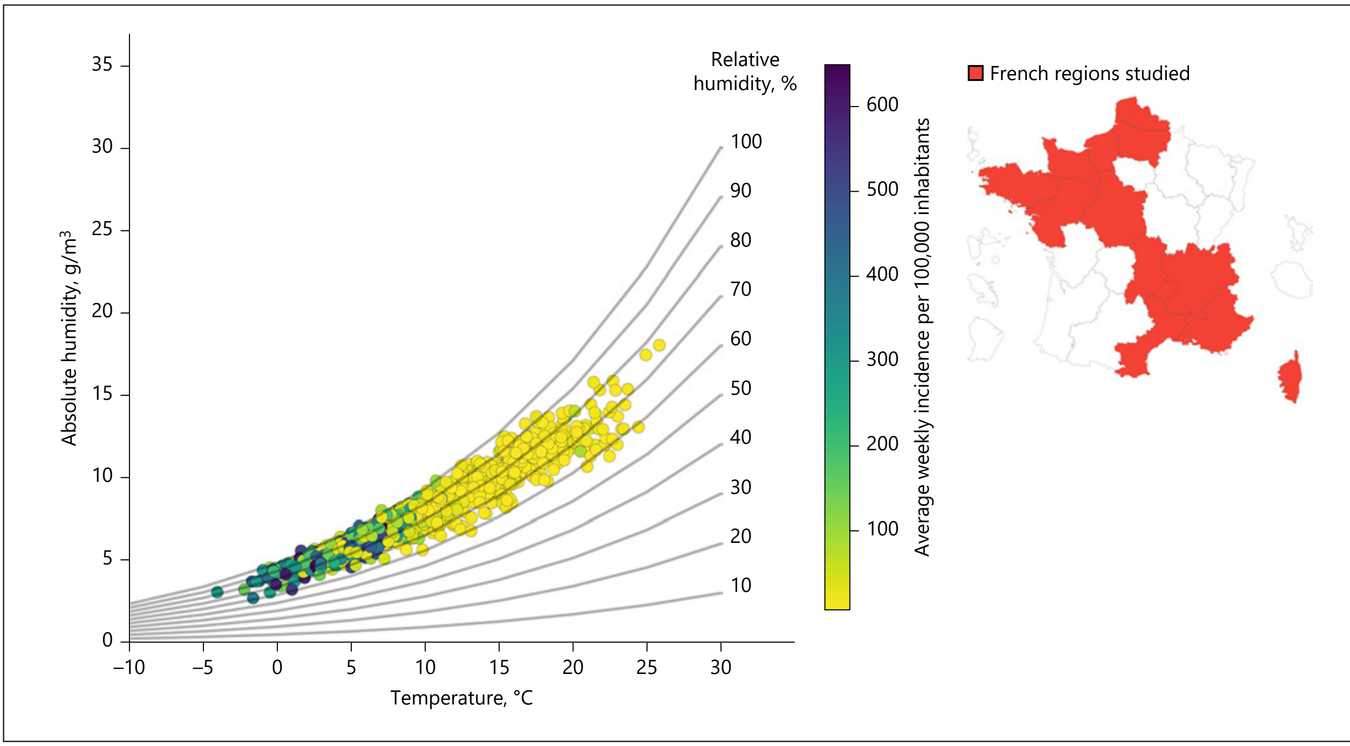


Fig. 4. Average climatic conditions (absolute humidity and temperature) associated with influenza virus reporting in 10 regions of France. The scatter plot shows the average climatic conditions (absolute humidity in g/m^3 and temperature in °C) of weeks with more than 5 influenza virus (IFV) cases per 100,000 inhabitants reported between 2016 and 2018 in different regions of France (Auvergne-Rhône-Alpes, Bretagne, Centre-Val de Loire, Corse, Hauts-de-France, Normandie, Nouvelle-Aquitaine, Occitanie, Pays de la Loire, and Provence-Alpes-Côte d'Azur). Each point represents a week with >5 laboratories confirmed influenza virus cases, and colours reflect incidence per 100,000 inhabitants. The black lines represent the variation of temperature and absolute humidity necessary to maintain specific relative humidity values (10–100%) in the air. Data of influenza virus incidence reported at the regional level originate from the Réseau Sentinelles (https://www.sentiweb.fr) [151], and climate data originate from the MeteoNet dataset (Meteo France) [152].

sure of water to the equilibrium vapour pressure, which equilibrium varies greatly with the temperature. In contrast, the absolute humidity is the actual mass of water vapour in the air, in g/m^3, irrespective of the temperature. As mentioned earlier, most experiments testing the influence of humidity on virus transmission considered only the relative humidity [7, 45–47]. However, recent reports highlighted that absolute humidity is a more likely accurate parameter for predicting influenza virus stability [144, 145]. While the debate is not completely closed [146, 147], it is likely that both parameters are equally valid in countries with a pronounced seasonality of epidemics, as most influenza virus cases arise in winter where the temperature is low, and therefore changes in relative humidity do not reflect important changes in the absolute humidity (Fig. 4). As pointed out by Marr et al. [147], it is also possible that the relative humidity might be a better predicting parameter of influenza transmission in indoor environments, where most transmissions likely happen, and correlate well with the outdoor absolute humidity. A recent attention has also been drawn to the influence of the air pollutants on influenza transmission [148]. It is interesting to note that the role of body fluids in the persistence of influenza virus gained a recent interest, as it was discovered that the mucus has a protective effect on the virus stability when dropped on banknotes [78]. It has also been reported that the mucus also plays a role in maintaining influenza virus infectivity when swallowed in the gastrointestinal tract [149] and in aerosols [150] (Fig. 4 [151, 152]).

Finally, regarding the molecular aspects influencing virus stability in the environment, we and others showed that influenza virus most external proteins, the haemagglutinin and the neuraminidase, are the main drivers of the environmental persistence of the virus [80, 153–155]. This could potentially explain how different strains of influenza virus or strains from different origins can have different persistence in the environment [82, 156]. It would be interesting to see if all enveloped viruses that have a class I membrane fusion protein and are transmitted through the environment, such as the coronaviruses or the Ebola virus, could follow the same principle.

Conclusion

In human history, several scientists understood early the link between the environment and the transmission of infectious disease. They understood that boiling water or avoiding close contacts and confined areas were efficient to prevent the spread of an infection, before the discovery of microorganisms. Since the discovery of the 1st virus a little more than a century ago, many more progresses were accomplished, describing the factors and mechanisms of virus persistence in water, in the air, or on surfaces. Here, we provide a comprehensive review on those historical discoveries that contributed to improve our strategy of virus control. Many more studies will be necessary in the future to describe in details the role of virus persistence in the environment in the virus epidemiology and ecology, or the relative importance of transmission by direct contact in comparison to airborne and waterborne transmission. Of particular interest, it will be essential to predict the impact of the climate change on the transmission of viruses and to observe how viruses adapt to their changing environment.

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Statement of Ethics

Ethical approval was not required for this project, as it is a synthesis of the scientific literature that did not involve research on human or animal subjects.

Conflict of Interest Statement

Prof. Jean-Claude Manuguerra is the editor-in-chief of *Intervirology*, Dr. Thomas Labadie serves as the managing editor, and Prof. India Leclercq is an editor of *Intervirology*. Mr. Christophe Batéjat has no conflicts of interest to disclose.

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T.L., C.B., I.L., and J.C. wrote and reviewed the manuscript.

Data Availability Statement

Data files and notebook used for data analysis of Figure 4 are available on the data repository Figshare (doi: 10.6084/m9.figshare.12672962).

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