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Survival of human enteric viruses in the environment and food

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Abstract

Human enteric pathogenic viruses can enter the environment through discharge of waste materials from infected persons, and be transmitted back to susceptible persons to continue the cycle of disease. Contamination of food with viruses may also promote disease outbreaks. A number of studies have investigated the survival characteristics of several enteric viruses in various environments and foodstuffs, to help explain the transmissibility of these pathogens. This review deals with published work on enteric virus survival on fomites, and in waters, soil, and foods; the results of these studies have illustrated the robust survival of viruses in these environments. Much information is lacking, however, especially for foodstuffs and soils, and no detailed information is available concerning the survival of noroviruses, the most significant foodborne type.

Keywords: Viruses; Environment; Food; Survival; Transmission

Contents

1. Introduction 442
2. Methodology used to study virus survival 442
3. Survival on fomites 442
4. Survival in water 444
   4.1. Seawater 444
   4.2. Fresh water 445
   4.3. Groundwater 446
   4.4. Drinking water 446
5. Survival in soil 446
6. Survival in food 448
   6.1. Crops 448
   6.2. Fresh vegetables 449
   6.3. Fruit 450
   6.4. Seafood 450
   6.5. Dairy products 451
   6.6. Other foods 451

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7. Conclusions. 451

Acknowledgements 452

References 452

1. Introduction

Transmission of a virus is dependent not only on its interaction with a host, but on its interaction with the environment outside of the host. Viruses outside a host may be regarded as inert particles, and, possessing no intrinsic metabolism, they do not require any nutrients to persist. Nonetheless, they possess a degree of robustness which allows them to remain infectious during the various conditions that they may encounter between one host and another. This is illustrated by the number of outbreaks of enteric viral disease attributable to water- or foodborne transmission [1,2].

The longer a virus can survive outside a host, the greater are its chances for transmission. These chances will be affected by various environmental conditions and factors as heat, moisture, and pH. These and other factors will vary in presence and extent among different environments. It would be advantageous to have complete knowledge of enteric virus survival in the environment, and the factors which influence it, to comprehend more fully the extent of the risks these pathogens pose, and to ascertain the means to break or curtail the chain of transmission. However, little work appears to have been performed to this end as yet, particularly for soil and food environments, and for fomites. The following review describes such work as has been published to date. In the review, the term "survival" indicates natural persistence of infectious viruses, i.e. when no process (such as heat, chemical disinfection etc.) has been deliberately applied to eliminate them. Only studies which have directly involved enteric viruses have been reviewed; no work using potential indicators such as bacteriophage has been included.

2. Methodology used to study virus survival

In general, studies to determine the potential for survival of viruses have been conducted using basic principles in common. A known number of infectious virus has been artificially introduced into a sample of water, soil, food etc., and the sample stored under conditions (e.g. temperature, moisture) relevant to those experienced naturally and for selected time periods. Then, viruses have been extracted from the sample and enumerated. There are various methods which can be used to extract viruses from food and environmental samples [3,4]; basically, they involve separating virus particles from gross solid material in the sample, and then concentrating them so that they may be delivered to a detection system. For enumeration of infectious viruses it is necessary to use cell culture in quantal format, e.g. plaque assay. The number of infectious virus remaining in the sample is compared with the number which was introduced, and statistical procedures can be performed to calculate any degree of decline.

3. Survival on fomites

Hospitals, homes for the elderly, and institutions housing small children can be subject to outbreaks of virus-associated diarrhea, but the role of surfaces and objects in the transmission of this has not been thoroughly investigated, save for a few studies. The results of theses studies indicate that enteric viruses can retain infectivity on environmental surfaces over prolonged periods.

In an early study, Kiseleva reported [5] that strains of poliovirus, echovirus and coxsackievirus remained infectious for 2 to >12 days on the surfaces of substances used in various household objects, i.e. painted wood, glass and cotton fabric, with greater survival being observed if the inoculum also contained coliform bacteria, protein, fat and dust particles. No precise results or experimental details were given, however.

Hands frequently contact environmental surfaces, and the potential for transfer of rotavirus between surfaces and hands was studied by Ansari et al. [6]. They inoculated fingertips with rotavirus Wa in a 10% fecal suspension, and monitored the survival of the viruses over 260 min. Sampling times were chosen to mimic the minimum period for inoculum drying (20 min) and the maximum period expected (260 min) between handwashings for institutional staff and those in their care. At 20, 60 and 260 min, approximately 57%, 43% and 7% of the initial virus number could be recovered. To measure transfer, fingertips inoculated with viruses were kept in contact with stainless steel discs for 10 s, then the discs were washed with buffer to recover any transferred viruses. At 20 and 60 min after fingertip inoculation, approximately 16% and 2% inoculated infectious virus could be transferred. When transfer was tested from disc to fingertip, almost identical results were obtained.

Contact between a contaminated and a clean hand 20 and 60 min after inoculation resulted in the transfer of approximately 7% and 3%, respectively. These findings showed that rotavirus could retain infectivity for several hours on skin, and could transfer in an infectious state to other surfaces.

A similar series of experiments was performed by Mbithi et al. [7,8], this time studying the survival and transfer of hepatitis A virus (HAV). They investigated the effect of relative humidity and air temperature on survival of hepatitis A virus on fecally contaminated stainless steel disks [7]. HAV survival was inversely proportional to the level of relative humidity and temperature. The virus survived better at 5 °C than at 20 or 35 °C. At 35 °C and 95% relative humidity virus was undetectable after 4 h, whereas at 5 °C and 95% relative humidity, 50% of infectious virus particle remained infectious. On fingertips [8], nearly 68% of the inoculated virus became undetectable after 20 min incubation, but, after 4 h, between 16% and 30% of the initially recoverable virus still remained detectable (the efficiency of recovery was calculated as 70%). As with the studies performed with rotavirus [6], drying appeared to account for the reduction in numbers of infectious virus. In hand/surface transfer experiments [8], approximately 25% viruses could be transferred from finger to disc and vice versa, but after 4 h only 1.6% surviving virus could be transferred from finger to disc and none the other way. The experiments were extended by examining what effect prolonged, increased contact pressure, and friction (turning the finger on the surface), had upon virus transfer: each mediated an increase. Transfer of HAV between hands could also be observed, apparently influenced by moisture. Moisture would mediate suspension of virus particles, and facilitate their movement between touching surfaces; drying would reduce this effect, as observed in the studies with rotavirus [6]. The persistence of HAV on environmental surfaces, and its ability to transfer to animate environments may be important factors in the spread of this virus, and explain in some part the unidentified mode of transmission in many investigated outbreaks.

Survival of human rotavirus on various non-porous (stainless steel, plastic) and porous (cloth, different types of papers) surfaces was studied by Sattar et al. [9]. The virus to be used for the contamination of the surfaces was suspended in fecal material, which was placed onto discs of the different materials, and held in glass chambers at various humidities and temperatures. In general, it was found that on non-porous surfaces viruses survived longer at lower temperatures (4 °C) and humidities (25–50%), with approximately 10% infectious virus remaining on the discs after 10 days, compared to less than 1% after 2 days at 22 °C and 85% humidity. Virus survival on porous surfaces was variable, but virus appeared to survive better on cloth than on paper.

Abad et al. [10] examined the survival of enteric adenovirus, HAV, poliovirus and rotavirus on fomites distinguished according to their porosity: porous materials used were paper and cotton cloth, and non-porous materials used were aluminum, china, glazed tile, latex and polystyrene. Three sets of environmental conditions were studied: 4 °C with high humidity (90%), 20 °C with high humidity (85%), and 20 °C with moderate humidity (50%). Viruses were inoculated onto the surfaces in phosphate-buffered saline (PBS) or fecal suspension. Each experiment was conducted over 60 days. It was found that HAV and rotavirus survived significantly ($P < 0.05$) longer on all fomites than enteric adenovirus or poliovirus. The former viruses survived up to 60 days on each surface reported, although declines in the titer of each of up to $3 \log_{10}$ were apparent; survival of poliovirus and enteric adenovirus was seldom recorded beyond 30 days, although on paper they did survive up to 60 days except in the presence of feces at 20 °C. Generally, the effect of feces was contradictory, enhancing or reducing virus survival depending on the fomes or virus type; the reason for this is not clear. It was shown, by drying of samples for a few hours in a flow cabinet, that dessication of viruses produced substantial and differential levels of inactivation of each virus type. On non-porous surfaces, HAV was most resistant to dessication ($P < 0.05$), followed by rotavirus, which outlasted enteric adenovirus and poliovirus ($P < 0.05$); on porous materials rotavirus was the most stable ($P < 0.05$), followed by HAV, poliovirus and enteric adenovirus, the latter being the most ($P < 0.05$) unstable virus. Resistance to dessication appears to be significant in determining the ability of enteric viruses to survive on fomites, and may account in some part for the seasonality of infections which has been observed for some virus types [11–13]. Ansari et al. [14] considered that seasonality of rotavirus infections may be due in part to the low relative humidity indoors during the cold periods, encouraging not only persistence of infectious virus on surfaces but also aerosolization of virus-laden dust particles. They reviewed information derived from experimental study of the survival of rotavirus in air: overall the information indicated that rotavirus could survive in air long enough to pose a risk of infection to persons or animals in the same environment.

Abad et al. [15] investigated the survival of astrovirus when dried on representative porous (paper) and non-porous (china) materials at 4 and 20 °C with high relative humidity $90 \pm 5\%$ over 90 days. The study was carried out with viruses suspended in PBS and a 20% fecal suspension. Astroviruses exhibited considerable persistence when dried on porous and non-porous materials. At 4 °C astrovirus was able to persist for 60 days dessicated on china, showing 4 and 5.3 $\log_{10}$ titer reductions in the presence and absence of fecal material, respectively. When dried on paper at the same

temperature, residual astrovirus infectivity was detected after 90 days, with reductions in titer of 4.3–4.5 log units. Much faster decay was observed at 20 °C, where astrovirus infectivity was detected only during the first 7 days after dessication, except when astrovirus was applied to paper suspended in PBS where residual infectivity was detected for 60 days. These results confirmed that astrovirus is able to survive on inert surfaces and fomites may play an important role in the secondary transmission of astrovirus diarrhea.

Noroviruses are a significant cause of outbreaks of gastroenteritis in institutions, and contamination of environmental surfaces may play an important role in the transmission of these agents in such settings. It has been estimated that over 30 million virus particles can be expelled in a single vomiting incident [16], and this could result in contamination of surfaces in the immediate area with large numbers of viruses via aerosolization of the vomit. Noroviruses have been detected on the surfaces of objects around patients during an outbreak of gastroenteritis in a hospital ward [17], and there is evidence [18] to suggest that prolonged survival of noroviruses may occur on fomites. Twelve days after an outbreak of norovirus infection in a hospital ward, two carpet fitters removed a carpet from one of the side rooms. Both men subsequently presented with the symptoms of norovirus infection and their only common exposure appeared to be the carpet, which they had had to handle extensively and vigorously in order to remove it. It had been vacuumed daily since the ward outbreak, and if it was indeed the source of the two fitters' illness, it would demonstrate considerable robustness in noroviruses.

Noroviruses cannot as yet be cultured in any known cell line, and this severely limits any study of their survival characteristics. However, feline calicivirus (FCV), a closely related virus which can be grown in cell culture, has been used as a surrogate. Doultree et al. [19] found that FCV dried onto glass coverslips and stored at 4 °C displayed a 4.75 log reduction over 56 days. Survival was lower at room temperature, but still prolonged, with virus numbers declining to undetectable levels by 21–28 days. At 37 °C no FCV was detectable on the coverslips after 1 day. The authors suggested that the effect of temperature on FCV survival may reflect the greater prevalence of norovirus infections in cooler seasons.

4. Survival in water

4.1. Seawater

Feline calicivirus (FCV) introduced into artificial seawater [20] exhibited a 20-fold reduction in titer after 1.5 h storage, but did not thereafter decrease over 24 h storage. The initial decline was attributed to the salt content of the water. Over 40 days storage in seawater [21], a 1–3 $\log_{10}$ decline in infectivity of FCV was observed at 4 and 10 °C, whereas at 20 °C there was a greater than 7 $\log_{10}$ reduction in infectious virus numbers.

Other enteric viral types have been shown to survive for many days in seawater. Loh et al. [22] inoculated samples of Hawaiian coastal waters, from the plume of a sewage outlet and 6.4 km further away, with poliovirus and incubated at 24 °C with continuous mixing for 4 days, to simulate natural conditions. They observed, in both samples, that the virus titer had slightly fallen after 1 day, but thereafter a rapid inactivation occurred, with an approximately 4 $\log_{10}$ reduction after 4 days. Under those experimental conditions the time required for 90% reduction (decimal reduction time) of poliovirus type 1 was 48 h, and complete inactivation was estimated to require 72–120 h. There was evidence to suggest that a virus-inactivating agent(s) of a biological nature was present in both the ''clean'' and sewage-polluted waters. A follow-up study [23] corroborated this evidence in that the antiviral activity of seawater samples was lost when they were subjected to boiling, autoclaving, or filtration.

A comparison of the survival of poliovirus and enteric adenoviruses (Ead) types 40 and 41 in seawater was performed by Enriquez et al. [24]. The studies were performed at 15 °C. The survival of poliovirus was shorter than the survival of either adenovirus type: the poliovirus titer was reduced by 3 $\log_{10}$ after 28 days, whilst Ead types 40 and 41 were reduced by 1.4 and 1.6 $\log_{10}$ respectively. The predicted times for 99% inactivation of each virus were 18 days for poliovirus, 85 days for Ead 40, and 77 days for Ead 41.

Another comparative survival study was performed using poliovirus and HAV [25]. Seawater from Spanish coastal areas was inoculated with the virus types and incubated at 5 and 25 °C for 30 days. Little inactivation of either virus was observed at 5 °C, and no difference in decay rates was observed. But at 25 °C decay was more pronounced, and there was a significant difference in the rates of inactivation of the virus types, with HAV being more stable than poliovirus. A pronounced decline was also observed in numbers of echovirus in these waters. It was considered that antiviral activity was due to bacteria: it was lost when the water samples were autoclaved or treated with antibacterial agents; a bacterium was isolated, after prolonged subculturing, which had the observed properties. These studies also found a marked difference in poliovirus survival in waters from Spanish coastal sites and from North Carolina coastal sites, with greater survival in the former waters. The overall antiviral action of waters will generally result from the dominant factors present, and it may have been that the anti-enterovirus factor of the Mediterranean samples was lacking in the North Atlantic samples. Another series [26] of experiments, however, did not reveal any

differences in the survival of enteric viruses (HAV and poliovirus) between seawater samples from North Carolina, California, and Hawaiian coastal waters. There was, nonetheless, a different rate of decline in titer for each virus, with poliovirus reducing by $4\log_{10}$ in approximately 1 week and HAV reducing by $4\log_{10}$ in about 4 weeks. The authors suggested that reported differences in survival of identical viruses in different seawaters might be a consequence of methodological differences. A standard survival protocol would allow this to be more fully investigated.

Microbial predation may not completely account for viral decline in seawater. Schwartzbrod and coworkers [27,28] observed a fall in the infectious titer of hepatitis A virus seeded into artificial seawater, which otherwise would have been sterile. The rate of decline was dependent upon temperature, with 90% inactivation after 11 days at 25 °C and 24 days at 19 °C; no decline was observed at 4 °C. Interestingly, HAV antigen declined far more slowly, with an estimated 90% loss after 178 days at 25 °C and 212 days at 25 °C [28]; further, detection of the genome by RTPCR could still be achieved after 232 days at 25 °C [27]. As free viral RNA is unlikely to persist much more than a few days in seawater [29], this indicates that inactivation in seawater might proceed by means other than disruption of the virus particle, possibly through an effect on receptor-binding proteins on the particle surface.

Ultraviolet light is highly efficient in the inactivation of poliovirus [30], and should strongly influence its (and probably all other enteric virus) survival in most natural environments. A series of tests [31] performed using Hawaiian marine waters seeded with poliovirus plus *Cryptosporidium*, *Giardia* and *Salmonella* and incubated in situ in experimental chambers showed an effect of sunlight on survival. When the chambers were covered, the time taken for 99% inactivation of poliovirus was 52 days; when exposed to light this time reduced to 20.6 days. Overall, the order of survival of the microorganisms in sunlight was *Cryptosporidium* > poliovirus > *Giardia* > *Salmonella*.

In in situ studies in North Carolina [32], experimental chambers inoculated with poliovirus were placed at water depths of 3–10 m, and kept there over 16 week periods which coincided with the seasons (e.g. Winter, Spring etc.). Survival times did not differ significantly between the two depths regardless of the season. Significantly ($P < 0.01$) higher survival times were noted only during the low temperatures (4–7 °C) of winter: there, they were 6.6–7.1 days for a 90% decline in infectious titre, as compared to 0.8–2.6 days for the other seasons.

4.2. Fresh water

Kutz and Gerba [33] reviewed studies of virus survival which had been published over the previous 30 years, which in turn had mostly been reviewed by Akin [34] and Sattar [35]. These studies had been performed with enteroviruses (polio-, echo- and coxsackieviruses). Summarizing the observations from these experiments and grouping them into freshwater sources gave mean viral inactivation rates of: tap water, $0.576\log_{10}\ d^{-1}$; polluted river water, $0.325\log_{10}\ d^{-1}$; unpolluted river water, $0.25\log_{10}\ d^{-1}$; impounded water, $0.374\log_{10}\ d^{-1}$; ground water, $0.174\log_{10}\ d^{-1}$. These rates are all less than $1\log_{10}$ per day, and indicated that viruses could survive in freshwater sources for prolonged periods of time. The authors recommended a standard protocol for virus survival studies, so that the effect of environmental conditions such as temperature could be analysed; so far, however, such a protocol has not been produced.

Hurst et al. [36] examined the long-term survival of coxsackievirus B3, echovirus 7 and poliovirus 1 in samples of surface freshwater collected from five sites of physically different character (artificial lake, small groundwater outlet pond, large- and a medium-sized river, small suburban creek). Survival was studied at temperatures of – 20, 1 and 22 °C. The average amount of viral inactivation was $6.5–7.0\log_{10}$ units over 8 weeks at 22 °C, $4–5\log_{10}$ units over 12 weeks at 1 °C, and $0.4–0.8\log_{10}$ units over 12 weeks at –20 °C. Several physical and chemical parameters (hardness and conductivity) appeared detrimental to virus survival. The turbidity of the water and suspended solids represented a beneficial influence for virus survival.

Rotaviruses may also be able to persist in freshwater for several days. In a study by Raphael et al. [37], it was demonstrated that a 99% reduction in rotavirus titer in seeded river water took approximately 10 days at 20 °C. At 4 °C the time taken for 99% reduction was 32 days. The difference was attributed to growth of bacteria and other microorganisms: when samples were filtered, no alteration in titer was observed throughout the test period of 64 days regardless of the holding temperature.

A more recent experimental study [38], using a tangential-flow, hollow-fiber system which allowed a continuous interchange of water through a chamber containing bacteria and viruses, examined their survival in two rivers – the fast-flowing Ottawa and the slower Rideau, which had a higher organic load. The virus types studied were poliovirus and hepatitis A virus. The authors reported little or no decay of hepatitis A virus in either river over the 48 days experimental period, but stated that poliovirus did show a decline; however, this latter conclusion is not apparent from the figures displayed in the report. The prolonged persistence of the viruses may have been in part due to the absence of indigenous microorganisms in the chamber itself, as it was unclear whether natural or sterilised water was used in the studies.

4.3. Groundwater

Land application of sewage effluents and sludges, as well as leachates from septic tanks, pose a risk of viral contamination of groundwater. A series of experiments to determine the potential for survival of enteric viruses in groundwater was performed by workers at the University of North Carolina [39]. Groundwater samples were taken from a deep well and seeded with poliovirus, echovirus or HAV, and incubated at 5 °C. Half of the water samples were sterilised by autoclaving before seeding. There was little inactivation of any virus over the 8 weeks of the experiment, regardless of the sterility of the samples. HAV was incubated at 25 °C in sterile and non-sterile samples also; in the former the virus survived without reduction in numbers over the 8 weeks of the experiment, but in the non-sterile samples there was a 99% inactivation by 2 weeks. Gordon and Toze [40] examining the effect of factors such as temperature, oxygen, nutrient levels and presence/absence of microorganisms on survival of poliovirus and coxsackievirus, concluded that the latter is the most influential factor in virus survival in groundwater.

4.4. Drinking water

Raphael et al. [37] observed no decline in seeded rotavirus numbers in tap water at 4 °C over 64 days, whereas at 20 °C the virus titer fell by approximately $2\log_{10}$ over the same period. The different effects at different temperatures might have been due to microbial growth, although the water had been chlorinated. The authors concluded that post-treatment contamination of potable waters with rotaviruses could lead to their widespread dissemination.

Enriquez et al. [24] studied the survival of poliovirus type 1, HAV and the enteric adenoviruses (types 40 and 41) in tap water at various temperatures for up to 60 days. Overall, they found that the enteric adenoviruses were more stable than the other viral types. At 4 °C, type 40 declined by less than $0.5\log_{10}$ and type 41 by almost 1 $\log_{10}$; however HAV and poliovirus decreased by 1.6 and 2.7 $\log_{10}$ respectively. The 99% reduction times established for these conditions were 41 days for poliovirus, 56 days for HAV, 92 days for adenovirus type 40, and 304 days for type 41, although the only significant differences were between both adenoviruses and poliovirus, and between type 41 and HAV. At 15 °C the titer of poliovirus declined by almost $3\log_{10}$ after 42 days (99% reduction time = 24 days), but adenovirus types 40 and 41 had only declined by 1.4 (99% reduction time = 87 days) and $1.2\log_{10}$ (99% reduction time = 124 days) respectively. At room temperature the titers of adenovirus types 40 and 41 decreased by nearly $2\log_{10}$ after 55 days, and poliovirus and HAV declined by 4 and $3.5\log_{10}$ after 3 and 50 days respectively. The predicted 99% reduction times were 60 and 84 days for types 40 and 41 respectively, 27 days for HAV and 11 days for poliovirus; only the differences between poliovirus and types 40 and 41 were significant, however.

Biziagos and coworkers [41] compared the survival in bottled mineral water of HAV and poliovirus type 1 at 4 °C and room temperature. Each virus persisted longest at 4 °C with little inactivation during more than 1 year. However, at room temperature, differences in virus survival were very discernible between the two viruses. At this temperature, infectious PV-1 was not detected after 300 days of exposure, while HAV was still infectious. The authors concluded that this prolonged persistence would increase the risk to consumers following contamination of mineral waters with enteric viruses.

Bosch and coworkers [42,43] studied the persistence of astrovirus in tap and marine water samples at 4 and 21 °C. They used a combination of infection of cultured $CaCo_2$ cells and RTPCR detection to compare numbers of infectious astrovirus before and after incubation in the samples for up to 90 days. After 60 days in dechlorinated tap water the decay of astrovirus infectivity was lower than $2\log_{10}$ at 4 °C and approximately $3.6\log_{10}$ at 20 °C, while after 90 days the titer reduction was approximately $3.3\log_{10}$ and $4.3\log_{10}$ respectively. In the seawater samples astrovirus showed a lower level of persistence, the virus inactivation after 30 days at 20 °C being the same as in tap water at 60 days, and achieving a $5\log_{10}$ reduction after 90 days. The authors suggested that the different rates of inactivation at 20 °C in tap and sea water could have been due to antiviral bacteria present in the latter samples.

5. Survival in soil

Because of the increasing emphasis which has been placed upon land application as a means of organic waste disposal, it has been considered important to evaluate the potential for survival of various pathogens in soils. Few studies however have concerned viruses, possibly because of the complexities involved in handling these agents and developing suitable extraction protocols for them.

An early series of experiments was performed by Bagdasaryan [44], using small (10 g) samples of soil inoculated with enteric viruses (poliovirus type 1, echovirus 7, echovirus 9 and coxsackie B3) and incubated under various conditions. Samples were taken at intervals over 170 days. Two soil types were studied, a sandy and a loamy soil; viruses appeared to survive somewhat longer in the former. The pH of the samples (adjusted by adding 10% $NaHCO_3$) affected survival in both soils: at pH 7.5 viruses could be isolated after 110–170 days, whereas at pH 5.0 survival times were between 25 and 60

days, depending on the virus type. The temperature of the soil had some influence on virus persistence: for example in loamy soil at pH 7.5 poliovirus and echovirus could be recovered after 110–130 days at 3–10 °C and 40–90 days at 18–23 °C. The moisture content of the soils had a marked effect: when air-dried sandy soil was inoculated, no viruses could be recovered after 25 days. No appreciable difference was seen in persistence of viruses in sterile and non-sterile soils.

Other studies have been performed in field settings. Tierney et al. [45] studied the persistence of poliovirus in sludge-amended soil, in Ohio. Here, small model vegetable plots were constructed in soil and stone filled boxes, planted with lettuce and radish and irrigated and cultivated as appropriate to normal practice. The plots were treated with virus-spiked effluent by flooding to a depth of 1 inch, and cultivated with a garden hoe 2 days afterward to till the sewage into the soil. Samples were taken regularly, treated by filtration, and the number of infectious viruses monitored by cell culture assay. The studies covered 123 days, and were conducted in spring, summer and winter seasons; temperature and rainfall were regularly recorded. The longest period of survival was during the winter, when virus was detected after 96 days. During the summer, the longest survival period was 11 days.

Damgaard-Larsen et al. [46] studied the survival of enteroviruses in sludge-amended soil under Danish winter conditions. In December 1975 they added coxsackievirus B3 to municipal sludges which were then placed on lysimeters containing heavy clay, neutral sandy, and acidic sandy soils. The sludge was dug in by spade, and samples taken each month until May 1976. The viral titer fell by 0.5–1 $\log_{10}$ per month, but some viruses could still be detected at the end of the experiment. The soil temperatures were not recorded but the air temperature varied between −12 and 26 °C during the duration of the test.

Hurst et al. [47] studied the survival of naturally occurring enteroviruses in sludge at a Texas sewage treatment plant and after subsequent land disposal of the solids after aerobic digestion. Samples were taken over several months. The moisture levels of the sludge-amended soils were recorded and compared with viral inactivation. The daily temperatures varied between 20 and 31 °C. No viruses were detectable in sludge solids which had been drying in a field for 3 months after land disposal, and results indicated that viral inactivation may have been directly related to loss of moisture in the sludge piles.

The Texas studies were extended [48] to study the effect of environmental variables and soil characteristics on survival of enteroviruses and rotavirus. Experiments were performed mainly using poliovirus. The main soil type studied was a loamy sand, and viruses were inoculated into 4 g samples of this soil and incubated under various conditions. Samples were assayed by dilution and direct inoculation. The presence of indigenous microorganisms deleteriously affected poliovirus survival, but this effect was not observed at low temperatures (1 °C), and poliovirus numbers remained unchanged throughout the 70 days of the experiment. Anaerobic conditions also prolonged virus survival. Poliovirus survival decreased as the soil moisture content increased up to 15% (the soil moisture saturation point), and then increased with the presence of additional amounts of liquid. This may have been due to differences in the extent and mechanisms of virus adsorption to soil under different moisture conditions, or to moisture-dependent differences in microbial growth rates. Survival of the various virus types was studied in nine soil types of differing physicochemical characteristics, and the results were analysed to determine which characteristics had the most marked effect. Factors such as the levels of organic matter and silt had no significant effect. Survival correlated significantly with virus adsorption to the soil and with pH, and a marginal significance ($P = 0.056$) was observed for resin-extractable phosphorous in the soils. Decreasing soil pH and phosphorous concentration may have increased adsorption. The authors suggested that a dilemma was posed by the observation that virus adsorption to soil may increase survival, in that adsorptive soils might otherwise be considered as the best sites for application of sewage wastes, as they would minimize virus transport to groundwaters.

Hurst [49] found that indigenous soil aerobic microorganisms significantly reduced survival of poliovirus, whereas indigenous soil anaerobic microorganisms did not. It was suggested that this may have been due not only to predation but to the production by the microorganisms of substances that interfered with adsorption of virus particles to soil particles.

Sobsey et al. [37] assessed the survival of poliovirus, echovirus and HAV in a variety of soils suspended in groundwater, secondary sewage treatment effluent and primary sewage treatment effluent. The samples used were both non-sterile and sterilised through autoclaving. When the samples were incubated at 5 °C viruses survived well in all cases, with 99% reduction times all calculated as being greater than 12 weeks. At 25 °C, a range of survival responses was observed. Generally HAV was more persistent than poliovirus or echovirus, affected to a lesser extent both by the higher temperature and soil microbial activity. In many of the soil types, such as loamy sand, clay loam and Bentonite clay, non-sterile and suspended in primary effluent, HAV 99% reduction times were greater than 12 weeks. Straub et al. [50] assessed virus survival in sewage sludge-amended soil under field conditions typical of the southwestern United States during winter and summer months. They seeded soil samples from freshly amended fields with poliovirus and buried them in containers 10 cm below

ground. Samples were taken every day for 7 days. Average soil temperature ranged from 15 °C in winter to 33 °C in summer; moisture decreased from 25% to 15% in winter and from 40% to less than 5% in summer. During the winter study, no inactivation of poliovirus was observed; in the summer study there was a $3\log_{10}$ reduction in the number of infectious poliovirus after 3 days, and no infectious virus was detected at 7 days.

Carrington et al. [51], reviewing the experiments of Tierney et al. [45], and Hurst et al. [47], produced a quantitative interpretation of viral decay rates from the data, and calculated decimal reduction times for poliovirus and total cytopathic enteroviruses in the sludge-amended soils. At the prevailing summer temperatures (19–34 °C) in these experiments, the decimal reduction rates were between 2.7 and 3.7 days; at the winter temperatures (13–26 °C) it was 24 days. Carrington et al. [51] likewise interpreted the data of Straub et al. [50]. Here, with a winter temperature of 15 °C, in the field at moisture levels of 25–15% the decimal reduction time was 92 days, and in the laboratory under evaporation conditions the decimal reduction time was 1.5 days. With summer temperatures of 27–33 °C, in the field at moisture levels of 3–40% the decimal reduction time was 1.2 days, and in the laboratory under evaporation conditions the reduction time was 0.4 days.

The above studies were mostly performed with North American soil types and indigenous climatic conditions, and information is lacking on persistence of viruses in soils and conditions pertaining to other countries. Carrington et al. [52] pointed out that in countries like the United Kingdom, mean soil temperatures seldom exceed 15 °C at 10 cm depth in summer, and are about 5 °C in winter. They suggested that viral decay rates would be slow under such conditions, with decimal reduction times from 24 days to over 100 days, but they also considered that cultivation of soil after sludge application would encourage viral decay by encouraging evaporation.

6. Survival in food

There have been many recorded outbreaks of viral infection attributed to the consumption of contaminated soft fruit, salad vegetables and other foods. Foodstuffs may become contaminated by contact with infected handlers, or by growth, irrigation or washing in contaminated waters. Although much information is lacking, that which is available gives some indication of the potential of enteric viruses to persist between preparation of foods and consumption.

6.1. Crops

Irrigation of crops with contaminated water or organic waste is a potential means of contaminating foodstuffs with enteric viruses, and studies have demonstrated that viruses can be transferred to the surfaces of vegetables and persist there for several days, following the application of sewage sludge or effluent.

The earliest published study was that of Grigor'eva et al. [53]. They grew tomatoes, white cabbage and sweet pepper in pots outdoors in the summer, and sprayed them with water inoculated with coxsackievirus A5, A7 and A14, various bacteria including *E. coli*, and a "coli bacteriophage". Leaves and fruits were sampled up to 20 days thereafter. The presence of coxsackievirus in the samples was determined through infection of newborn mice. Highest survival of each pathogen on the leaves of each plant appeared to be tomatoes > peppers > cabbage, which was tentatively ascribed to the smoother surfaces of the last of these providing less protection from dessication. Coxsackievirus remained infectious between 15 and 18 days on tomato leaves, between 7 and 10 days on sweet pepper leaves, and 3–4 days on cabbage leaves, depending on the type (or possibly due to differences in infectious titer at the start of the experiment). The results of the fruit samples were not thoroughly reported; apparently coxsackievirus A5 could be recovered up to 8 days on tomatoes, and coxsackievirus A14 up to 10 days on sweet peppers. *E. coli* survival times were in all cases shorter than coxsackievirus, whereas bacteriophage survival correlated well with that of the enteric viruses.

Larkin et al. [54] and Tierney et al. [45] used small field plots to which poliovirus-seeded sewage was applied by spray or flood irrigation, shortly before or shortly after planting of lettuce and radish seeds. After spraying, visible particulate material was retained on the plant surfaces of all sludge-irrigated vegetables and persisted until the crops were picked. After spray irrigation of sludge or effluent [54], poliovirus was recovered from mature vegetables for up to 36 days in one experiment, although a 99% loss in detectable viruses was noted during the first 5–6 days of it, and up to 14 days in another experiment conducted somewhat earlier in the growing season. In the latter experiment, extensive runoff occurred during thunder showers and viruses were probably removed from the vegetables into the drainage system. After flooding with effluent, polioviruses were detectable up to 23 days [45]. The above tests were performed using fairly high titers of virus (2.5×10^8–1×10^{13} infectious units l^{-1}). Ward and Irvine [55] considered that these concentrations could influence the length of time during which infectious virus persists on crops at sufficient concentration to be detected; accordingly, they used poliovirus and adenovirus in their survival study at titers 5.1×10^2–2.6×10^5, which, they stated, were similar to those commonly found in wastewater before storage. They found that poliovirus survived on celery, spinach, lettuce and tomatoes for 4, 13, <2 and 6 days respectively, following irrigation of these crops for 2 h with seeded wastewater.

As previously observed [54,45], there was a rapid initial decline of >90% in recovered infectious virus in all cases: this possibly may have been due to drying of the crops, or the effect of sunlight. Solar radiation will have a considerable negative influence on the persistence of viruses on irrigated crops, as demonstrated by Kott and Fishelson [56] comparing survival of poliovirus seeded on to parsley over 24 h indoors and outdoors.

Badawy et al. [57] examined the potential for survival of poliovirus type 1 and rotavirus SA-11 on sewage-irrigated grasses. Few experimental details were given, but it was stated that the viruses survived from 8 to 40 h under field conditions. Survival was influenced by temperature: in winter poliovirus and rotavirus were reduced by 1.5 $\log_{10}$ and 2.3 $\log_{10}$ in 24 h respectively, and during the summer poliovirus was reduced by 3 $\log_{10}$ within 8 h.

There may be factors within sewage which limit virus survival. Kott and Fishelson [56] found that poliovirus persisted longer when seeded onto tomato and lettuce plants in phosphate-buffered saline than when seeded in oxidation pond effluent; this may perhaps have been due to microbial activity.

6.2. Fresh vegetables

Once on foodstuffs such as vegetables, viruses may persist under normal storage conditions over the times usual between purchase and consumption. Ward and Irving [55] harvested celery and spinach immediately after 2 h irrigation with poliovirus-seeded wastewater, and stored 0.5–3.0 kg samples at 4 °C in a humid atmosphere. Virus survival of at least 76 days on celery and 55 days on spinach was observed. There was an approximately 2 log decline in recovered infectious virus in each case. Bagdasaryan [44] reported that poliovirus, echovirus and coxsackie B3 virus could survive on lettuce, tomato, cucumber and radishes for up to 10–15 days at refrigeration temperature with less than 1 log reduction in titer, whilst at room temperature the reduction over the same period was 2–2.5 log.

In another early study, Konowalchuk and Speirs [58] determined the survival of coxsackie B5 virus, poliovirus type 1, echovirus 7, reovirus 1 and adenovirus type 7a on celery, lettuce, carrots, green peppers, tomatoes and radishes. Viruses were inoculated onto the vegetable surfaces in water or dilute feces, and the samples were stored covered or uncovered at 4 °C for up to 8 days. It was found that, whereas on the covered samples there was no loss of virus titer, on uncovered samples, where the inocula could dry, the titer declined to 30% of the original or less after 1 day, and no infectious virus could be recovered after 4 days. The presence of feces delayed these effects, by reducing the evaporation of the inoculum. Coxsackievirus was inoculated onto radish and carrot in water, dilute feces or raw feces, and after 5 days could be recovered at <1%, 4% and 7% of the original inoculum, respectively. It was considered that vegetables with moist surfaces such as lettuce and celery were best suited for virus survival. Lettuce was also the best vehicle for rotavirus survival in a series of experiments by Badawy et al. [59]. Here, simian rotavirus SA-11 was used as a model, due to the difficulties in propagating human rotavirus types. Small pieces (5–15 cm^2) of lettuce, radishes and carrots were inoculated with virus suspensions, either by 0.1 ml drop or by immersion in 200 ml, then stored in covered and uncovered containers at 4 and 25 °C. Samples were collected and analysed over 30 days. Rotavirus SA-11 survived on lettuce, radishes and carrots for up to 30, 30, and 25 days respectively at refrigeration temperature after direct inoculation; at room temperature however the survival periods were 25, 4, and 15 days respectively. Greater virus inactivation occurred if viruses were inoculated by immersion than by drop, but covering the samples made no difference. The recovery of inoculated virus was lower from carrots than from the other two vegetables: this may have been due to an antiviral effect of the foodstuff, or elution may not have been so effective with these vegetable samples.

Kurdziel et al. [60] inoculated Webb's lettuce, green onions, white cabbage, strawberries and raspberries with poliovirus, and stored the samples at 4 °C over 15 days. A 1 $\log_{10}$ decline in infectious virus was observed on lettuce and white cabbage, but on green onion there was no significant decline. The differences were probably due to the flat surfaces of lettuce and cabbage providing less protection against dessication than green onion. Croci et al. [61] obtained similar findings with HAV on lettuce, and also evaluated HAV survival on carrot and fennel. On the latter vegetables, a more pronounced decline in HAV infectivity was observed, with complete inactivation of HAV by day 4 for carrot and by day 7 for fennel. It was considered that this may have been due to the presence of antimicrobial substances in these vegetables. Overall however, the results of Kurdziel et al. [60] and Croci et al. [61] provide a strong indication that infectious enteric viruses may persist on fresh fruit and vegetables for several days under conditions commonly used for storage in households.

Bidawid et al. [62] investigated the effect of various modified atmospheres on the survival of HAV on lettuce. Inoculated lettuce was stored at room temperature and 4 °C for up to 12 days in ambient air and under various modified atmospheres. The lettuce samples were stored in heat-sealed bags with the following percentages of gas mixtures (CO_2:N_2): 30:70, 50:50, 70:30, and 100% CO_2. Only at 70% CO_2 at room temperature was a significant decline in virus survival observed. As most commercially distributed lettuce is stored under lower CO_2 concentrations, this measure may not provide protection against transmission of contaminating viruses.

6.3. Fruit

Two published studies are available on the potential for persistence of enteric viruses on fresh fruit. Konowalchuk and Speirs [63] studied the survival of coxsackie B5 virus, echovirus and reovirus on strawberries, cherries, and peaches at 4 °C in uncovered or closed containers containing water. The study was performed by inoculating viruses in a drop of suspension onto the surfaces of individual fruits. The tight closure of the containers with water ensured a degree of humidity. In half of the experiments, the viruses were inoculated in dilute feces, to simulate some natural contamination conditions. The experiments were conducted for up to 6 days. In general, humid conditions prolonged virus survival, for example 100% of seeded coxsackievirus B5 could be recovered after 6 days humid incubation of cherries, whereas <1% could be recovered after 6 days dry incubation. The presence of feces greatly enhanced virus survival in all cases, probably by retarding dessication. Virus survival on strawberries was lower than the other two fruits, probably due to antiviral substances in this fruit, with survival of each virus on cherries and peaches being similar. Reovirus was less persistent than the other two types on all the fruit samples, or else the efficiency of its recovery was lower. The authors concluded that natural irradiation and dessication, in combination with natural antiviral substances which may be present in many fruits, may reduce virus infectivity to a minimal risk.

Lee and Kop [64] looked at virus survival on pieces of cut papaya, a popular food sold by street vendors in tropical countries. They used poliovirus as a model, and observed a rapid initial decline in infectious titer: after 1 h incubation in an ice box between approximately 10% and 30% of seeded poliovirus could be recovered, depending on the ripeness of the fruit. The experiments were conducted for 6 h, but no further decline was noted. They concluded that if tropical fruits like papaya that are large and have skins which cannot be removed by peeling are contaminated by enteric viruses during preparation, then there is a risk that sufficient viruses may remain infectious through to the point of consumption by street purchasers. The inactivation observed was probably due to antiviral effects of substances in the fruit exudates. Similar effects have been noted [65–68] and attributed to plant polyphenols such as tannin; these effects have however been shown to be reversible [68] and may not provide protection against viral infection.

O'Mahony et al. [69] examined the survival of rotavirus in an artificially contaminated filtered fruit juice (pH 2.98), infant formula milk and lettuce during storage for 3 days at 4 °C. An approximately $3\log_{10}$ reduction in infectious rotavirus titer was observed in juice after 3 days. This indicates that rotavirus has the potential to survive well in low pH fruit juice at refrigeration temperatures.

6.4. Seafood

Hejkal and Gerba [70] looked at the survival of poliovirus in blue crabs, by allowing the crabs to accumulate polioviruses from artificial seawater at 25 °C for 4 h, then placing the crabs in clean water at 15 and 25 °C for up to 6 days. At 15 °C virus was still detectable up to 6 days in the hemolymph and digestive tract and up to 3 days in the meat. The loss of titer was 96.4% in the digestive tract, 98.2% in the hemolymph, and >98% in the meat after 6 days. At 25 °C, loss of virus proceeded at a greater rate: no virus was detected in the meat or digestive tract after 2 h, the hemolymph had lost 99.7% of its original titer in 20 h, and 1 pfu was recovered from the hemolymph of one crab after 44 h. This study showed that crabs living in contaminated water can be expected to accumulate some viruses, and retain them for several days depending on the water temperature. However, it was also demonstrated that cooking abolished virus infectivity in the crabs within a few minutes.

Persistence of poliovirus in whole and shucked oysters (*Crassostrea virginica*) stored at 5 °C was monitored by Tierney et al. [71]. Shellfish were contaminated with virus either through exposure to virus-contaminated seawater or by direct inoculation, before storage for up to 77 days. In all samples, viruses were detectable throughout storage. The authors considered that, as the longest time normally employed between harvesting and consumption is around 28 days, if infectious enteroviruses are present in oysters at the time of harvesting, they will still be present when the shellfish are purchased for processing in the home or food establishment.

DiGirolamo et al. [72] studied the survival of poliovirus in chilled, frozen and processed Pacific (*Crassostrea gigas*) and Olympia (*Ostrea lurida*) oysters. Samples of whole oysters were contaminated with 10^4 pfu ml^{-1} of poliovirus and were then stored. Olympia oysters are normally eaten raw, so they were stored refrigerated at 5 °C assayed for virus at 0, 5, 10, 15, 20, 25 and 30 days. Pacific oysters are normally cooked, and they were frozen at −17 °C, and assayed for virus at 0, 2, 4, 6, 8, 10 and 12 weeks. After 15 days of storage at 5 °C, infectious virus in the Olympia oysters was reduced by 60%; after 30 days 13% of the virus was still infectious. In frozen Pacific oysters, the titer of poliovirus was reduced by less than 10% after 4 weeks; after 12 weeks only 10% infectious virus remained. Greening et al. [73] determined the survival of poliovirus in artificially contaminated fresh and frozen green – lipped mussels (*Perna canaliculus*) after 2 days storage at 4 °C, and after 7, 14 and 28 days storage at –20 °C. After 2 days at 4 °C, 81% infectious virus remained in the mussels. Infectious virus declined to 66%, 53% and 44% of the original number

after storage at –20 °C for 7, 14, and 28 days, respectively. The authors considered that the ability of enteric viruses to survive in chilled and frozen shellfish represents a potential health risk and is significant both to the shellfish industry and public health agencies.

6.5. Dairy products

Tiron [74], having observed the presence of enteroviruses in samples of dairy foods (milk, yogurt and cheeses) from farms and processing plants in Romania, studied the potential for survival of these agents using artificially inoculated samples. In pasteurised milk and boiled milk, poliovirus and coxsackievirus B5 could survive for at least 90 days at 4 °C; at 25 °C survival was 15 and 30 days for each virus type respectively. Echovirus was observed to survive for up to 120 days in raw milk. Yogurt held at 4 °C supported the survival of poliovirus and coxsackievirus B5 for 90 days, and echovirus for at least 120 days. In cottage cheese each virus could survive up to 120 days. In these experiments the viruses were inoculated at 10^4 tissue culture infectious $dose_{50}$ ($TCID_{50}$) units; when inoculation was performed using 10^2 $TCID_{50}$ units survival times were often lower, although this varied between virus type and foodstuff. Kiseleva [5] reported that strains of poliovirus, echovirus and coxsackievirus remained infectious for 3–5 days in milk, dairy products and bread, but no precise results or experimental details were provided. Cliver et al. [75] showed that poliovirus inoculated into cheese could persist over 7 months with less than one log decline in titer, but considered that the cheese-making process was sufficient to control any viral contamination which might be introduced into raw milk.

6.6. Other foods

Herrmann and Cliver [76] observed a 2–3 $\log_{10}$ reduction in titer of coxsackievirus A9 inoculated into raw ground beef and stored at 4 °C for 14 days. This is of course a longer storage period than would be used either domestically or commercially, but the persistence of the enteric virus indicates that raw ground beef contaminated by an infected handler could be a vehicle for transmission if eaten uncooked, for example in steak tartare.

Cliver et al. [77] studied the potential for enteric virus survival in low moisture foods developed for space flights, mimicking contamination by a food handler during final packaging. The foods tested were from a wide range of types (including for example bacon, cheese sandwiches, spaghetti and banana pudding). Each foodstuff was freeze-dried, inoculated with viruses, sealed under vacuum in plastic pouches, then stored at room temperature, 5 or 12 °C. The enteric virus types were reovirus, poliovirus types 1 and 2 and echovirus type 6. Although it appeared that reovirus was not capable of persisting after 1 day in these foods, the enteroviruses showed great stability, persisting up to 2 weeks at room temperature and up to 2 months at refrigeration temperatures. There were interactions between temperature, pH, protein and salt content upon virus survival in the low moisture foods; these were complex and not easily interpreted, but may also be operational in other foodstuffs.

It would be interesting to examine survival of viruses in foods such as salted peanuts and crisps, such as are often consumed communally and in public settings (bars, restaurants, functions etc.) where several people can consume a shared portion. However, this has not been done yet.

7. Conclusions

Although some information on virus survival has been acquired, particularly for the water environments, much is lacking. No study has comprehensively included all enteric virus types, even those which can be readily propagated in cell culture, and there is no hard information on such important viral agents as noroviruses or hepatitis E virus. Studies with noroviruses must await the development of suitable propagation techniques, and it is hoped that the appropriate bodies take up recommendations to this end [78]. It will be most advantageous if future virus survival studies are harmonised, i.e. by the use of common features such as numbers of infectious virus used per sample, similar sampling times, and a standard procedure for statistical analysis of the results. This will facilitate the acquisition of comparative data for all virus types studied.

A thorough assessment of the fate of viruses in soils, especially those to which sewage has been applied, is necessary to determine whether any agricultural controls currently employed are fully effective. For example, the UK Code of Practice 1989 [79] prohibits planting of crops soon after application of sludge, but the amended soil studies described above indicated a potential for several months survival of viruses in soils in cool climates such as those found in the British Isles, and there may therefore be possibility of entry into the food chain thereby.

Persistence of viruses on foods may be challenged by various disinfection techniques, and the survival times of each virus type on different foodstuffs will provide baseline information to assess the efficacy of these techniques. Again, more studies are necessary to acquire this information comprehensively on all enteric virus types.

This review has summarized existing information; it is hoped that it will be useful in planning work to fill in the gaps.

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