Update Le point

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Human viruses in sediments, sludges, and soils*

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Recent studies have provided a greater understanding of the movement of viruses in the environment by their attachment to solids. These studies have focused on solids-associated viruses present in wastewater discharged into the ocean and on viruses in sludge and wastewater that may be retained in soil following their land disposal. Such ocean or land disposal of wastewater and sludge may result in a discharge of one or more of 120 human enteric virus pathogens including those causing poliomyelitis, viral hepatitis A and acute gastroenteritis.

Solids-associated viruses in effluents discharged into coastal waters accumulate in bottom sediments, which may contain 10 to 10 000 more virus per unit volume than the overlying seawater. Solids-associated viruses resuspended by water turbulence may be transported from polluted to distant non-polluted recreational or shellfish-growing water. Transmission of viruses causing hepatitis or gastroenteritis may result from contact by bathers or swimmers with these viruses in recreational waters, or from ingestion of raw or improperly cooked shellfish in which the solids-associated virus had been bioaccumulated.

The land disposal of sludge and wastewater has a potential of causing infections in farm workers, contamination of crops, pollution of raw potable water sources or infiltration of ground water. Viruses retained on soils can be released by rain water and may contaminate ground water through lateral and vertical movements.

More than 120 different virus types are known to be excreted in human faeces by infected persons, whether or not they manifest illness. These viruses belong to various groups including enteroviruses (polioviruses, coxsackieviruses, echoviruses and hepatitis A virus), reoviruses, adenoviruses, and parvoviruses (adeno-associated viruses) (Table 1).

The frequency of isolation and quantity of virus recovered from sewage depends not only upon the infections caused by normally occurring viruses and those induced by oral poliovirus vaccine, but also upon the efficiency of the recovery procedures. The time of

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Table 1. Human enteric viruses that may be present in polluted water

Virus group	No. of types	Disease caused
Enteroviruses:		
Poliovirus	3	Paralysis, meningitis, fever
Echovirus	31	Meningitis, respiratory disease, rash, diarrhoea, fever
Coxsackievirus A	23	Herpangina, respiratory disease, meningitis, fever
Coxsackievirus B	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia
New enteroviruses:		
Types 68–71	> 4	Meningitis, encephalitis, respiratory disease, acute haemorrhagic conjunctivitis, fever
Enterovirus type 72 (hepatitis A virus)	1	Viral hepatitis A
Gastroenteritis (Norwalk) virus	2	Epidemic vomiting and diarrhoea, fever
Rotavirus	4	Epidemic vomiting and diarrhoea, chiefly of children
Reovirus	3	Not clearly established
Adenovirus	37	Respiratory disease, eye infections, gastroenteritis
Parvovirus (adeno-associated virus)	3	Fever, rash, aplastic anaemia

sample collection (day and season) and water use within a community are also important factors (1). Average virus recoveries in raw sewage are about 100 plaque-forming units (PFU) per litre in the USA (2). Viral recoveries from sewage in other countries have generally been higher (e.g., India, $10^{2.3}$ – 10^4 ; Israel, 10^4 – 10^6 ; South Africa, $10^{4.9}$) (3–5).

This article describes the public health hazards associated with discharge to ocean outfalls or land surfaces of virus-laden sewage. Special attention is given to the protection and transport of viruses from polluted to non-polluted recreational and shellfish waters, and ground-water contamination resulting from the retention and movement of virus through soils.

HUMAN VIRUSES IN ESTUARINE SEDIMENTS

Many communities discharge their wastes into estuaries or coastal waters. The world's oceans receive billions of litres of treated and untreated domestic and industrial wastes and sewage sludges, as well as polluted surface waters. Coastal waters of the United States receive more than 30 000 million litres of municipal sewage in a single day. A number of human enteric viruses may be present even in treated effluents. A high percentage of viruses are associated with solids in sewage effluents and thereby protected from chlorine inactivation (6). The fate of viruses discharged into coastal water is shown in Fig. 1.

Viruses associated with large particles ($>6\ \mu\text{m}$) soon leave a water column and settle into bottom sediments, while viruses adsorbed on smaller particulates or colloids ($<3\ \mu\text{m}$) tend to stay suspended in the water for a longer time. When suspended solids-associated viruses settle out of a water column, they accumulate in a loose, fluffy layer over the compact bottom sediment. Sediments in coastal waters serve as a reservoir from which virus can be released into a water column by storm action, dredging, and boating. Viruses from fluffy sediments can be more easily resuspended by mild turbulence or water movements. These resuspended viruses from polluted waters can be transported to distant

Fig. 1. Fate of viruses in coastal systems.

(From Melnick, J. L. & Gerba, C. P. *Public health reviews*, 9: 185–213 (1980))

non-polluted recreational and shellfish waters, depending on tidal currents, water circulation patterns and prevailing winds.

Quantity of viruses in sediments

The magnitude of health hazards associated with the discharge of virus-laden sewage wastes into marine waters depends upon the quantities of virus released and their survival in water, sediment, and on suspended solids. Recent advances in methods for concentration of virus from marine waters and sediments have made field studies on the occurrence and distribution of viruses possible. Enteroviruses and rotaviruses are concentrated from >400-litre volumes of turbid seawater or as much as 1000 grams of sediment, with an efficiency of about 50%.

Reports on virus quantities in estuarine sediments have been few. Data given in Table 2

Table 2. Recovery of enteroviruses from estuarine and marine sediments

Type of sediment	Site location	Virus concentration (PFU per 100 litres)	
		Water	Sediment
Marine sediment ^a	Hollywood	0.3	3160
	Miami	0.5	2160
	Miami Beach	7.3	9830
Estuarine sediment ^b	(1) Coastal canal community along the Texas coast (1 m from sewage outfall)	160.0	2000
	(2) 300 m from sewage outfall	90.0	2080

^a Data from Gerba, C. P. et al. In: Litchfield, C. D. & Seyfried, P. L., ed. *Methodology for biomass determination and microbial activities in sediments*, American Society for Testing and Materials, 1979, pp. 64–74.

^b From Gerba, C. P. et al. *Marine pollution bulletin*, 8: 279–282 (1977).

Table 3. Recovery of enteroviruses and rotaviruses from Galveston Bay samples

Virus group	Sample	No. examined	% positive	No. of PFU ^a
Enterovirus ^b	Water	35	14	3–12
	Compact sediment	35	6	7–10
	Fluffy sediment ^c	15	47	39–398
	Suspended solids ^d	18	72	4–40
Rotavirus ^e	Water	31	16	119–1000
	Compact sediment	8	12	1200
	Fluffy sediment ^c	15	40	800–3800
	Suspended solids ^d	18	50	1800–4980

^a Plaque-forming units of virus estimated for 250 litres of water and 1000 grams of compact sediment.

^b From Rao, V. C. et al. *Applied and environmental microbiology*, 48: 404–409 (1984).

^c Obtained by filtering 40 to 60 litres of water containing fluffy sediment (loose upper layer of bottom sediment disturbed and suspended in water near the bottom).

^d Obtained by filtering a similar volume of water containing suspended solids near the surface.

^e From Rao, V. C. & Melnick, J. L. *Applied and environmental microbiology* (in press).

are based on a volume–volume comparison of virus concentration in water and sediment and probably do not represent a true comparison of the concentration differences. The data show an approximately 10 to 10 000-fold higher concentration of virus in sediment than in the overlying water at sites in both Texas and Florida.

Our own studies in Galveston Bay were designed to determine and compare the quantitative distribution of naturally occurring enteroviruses and rotaviruses in water, suspended solids, fluffy sediment, and compact sediment (7). Data presented in Table 3 indicate that virus was most often attached to suspended solids (<3µm size); 72% of these samples were positive for enteroviruses compared to only 14% of the water samples. Fluffy sediments yielded enteroviruses in 47% of samples, while only 6% of the compact bottom-sediment samples tested were positive for virus. Rotaviruses were associated with one half of the suspended solids samples, more than in any of the other samples. Although a greater number of enteroviruses were recovered from fluffy sediments than from suspended solids (39–398 PFU per 250 litres), the reverse was true of rotaviruses (800–3800 PFU per 250 litres versus 1800–4980 PFU from suspended solids).

Enteroviruses are recovered from estuarine sediments by a simple method (8) which consists of the following steps: (i) the sediment is shaken with 3% beef extract solution containing sodium nitrate (this salt has the ability to disrupt hydrophobic interaction between the virus and sediment); (ii) the sample is centrifuged and the sediment deposit is discarded; (iii) the virus in the beef-extract supernate is concentrated to a small volume by adjusting the sample pH to 3.5 and flocculating the organic matter along with the virus. The floc is separated by centrifugation and dissolved in sodium phosphate, thereby releasing the virus from the floc.

Survival of viruses in sediments

Enteric viruses are known to attach themselves readily to the solids in water, and several laboratory experiments have yielded data indicating prolonged survival of these solids-associated viruses. Potential adsorbents found in marine water include sand, clays (montmorillonite, kaolinite, bentonite, illite), aquatic life forms (algae, bacteria), silts, and sediments. Data on the survival of viruses in estuarine sediments have been provided (9). Echovirus 1 and coxsackievirus B3 had the longest survival time, 18 days or greater; poliovirus, 14 days; and coxsackievirus A9, 4 days. In the sample containing seawater

alone, coxsackievirus A9 was undetectable after 2 days, and the other viruses persisted for 4, 6 and 10 days, respectively.

In a recent study, we examined the survival of poliovirus and rotavirus seeded in seawater containing fluffy sediments and suspended solids collected from Galveston Bay. Since these two sediments have the greatest chances of being transported to distant areas, data on the survival of viruses associated with them are especially significant. Both viruses survived longer when associated with solids, whether suspended or sedimented. Both viruses could be detected in the fluffy sediment and suspended solids even on the nineteenth day, but they could not be detected beyond the ninth day in seawater alone (7).

Studies on the survival of enteroviruses under field conditions were also carried out at two sites near a sewer outfall in Galveston Bay. Cellulose dialysis tubes filled with seawater or a mixture of sediment and seawater, and seeded with known numbers of polio- and echoviruses were held in a plastic bait bucket with perforated sides at a depth of 1.5 metres. The quantity of virus remaining in the samples was determined at one-day intervals for seven days. Both polio- and echoviruses were inactivated much faster in seawater alone than in the presence of sediment and confirmed the protective effect of sediment on the viruses (Fig. 2).

Although the exact mechanism of increased virus survival while adsorbed to solids and sedimentary particulates is unknown, we may speculate. Adsorbed viruses offer less surface for interaction with inactivating substances, a feature which may result in greater protection of virion structures. Degradation of a virus particle might involve a reorientation or freeing of the viral capsid, and the energy binding the virus to the particulate matter would have to be overcome, and higher temperatures or greater concentrations of chlorine would be required than if the virus particles were free.

Fig. 2. Survival of poliovirus 1 and echovirus 1 in polluted estuarine water in Galveston Bay.

(---○---) poliovirus suspended in seawater; (—●—) poliovirus suspended in seawater and sediment; (---○---) echovirus suspended in seawater; (—●—) echovirus suspended in seawater and sediment; Log N_t/N_0 = the log of virus concentration at the time of sampling divided by the virus concentration at time 0 hour.

(From Smith, E. M. et al. *Applied and environmental microbiology*, 35: 685-689 (1978))

ation or freeing of the viral capsid, and the energy binding the virus to the particulate matter would have to be overcome, and higher temperatures or greater concentrations of chlorine would be required than if the virus particles were free.

Significance of virus-sediment association

Solids-associated viruses transported to swimming beaches and shellfish-growing waters may result in virus transmission to humans.

Transmission of enteric viruses by recreational water sources. Recreational use of marine waters may play a role in the transmission of enteric viral disease. The US Environmental Protection Agency recently completed a 7-year study on epidemiological-microbiological water quality and gastrointestinal symptoms among bathers. Swimming-associated gastroenteritis, primarily in children, was associated with the bathing quality as measured by enterococcus and *Escherichia coli* densities. Gastroenteritis followed after short incubation periods and was characterized by acute onset, a short period of relatively benign symptoms, and no sequelae, although in some individuals the symptoms were

disabling enough for the patients to stay at home, remain in bed, or seek medical advice. The association between illness and the presence of only 10 *E. coli* per 100 ml suggested that the agents responsible for the illness were highly infectious, were present in sewage in large numbers, and survived much longer than *E. coli* in a marine environment. These characteristics, along with the clinical aspects of the illness, suggested a viral etiology (10).

Role of shellfish in the transmission of enteric viral disease. Shellfish (oysters, mussels and clams) filter out suspended food particles from water pumped through the shell cavity during feeding. Shellfish can filter from 4 to more than 20 litres of water per hour. Along with the food particles, bacteria and viruses are trapped in the mucous sheaths and pass into the alimentary tract. Since entire shellfish are often consumed raw or after inadequate cooking, they can act as passive carriers of human pathogenic viruses.

Viral disease outbreaks attributed to shellfish. Viral hepatitis A and gastroenteritis are viral diseases known to be transmitted by shellfish. These outbreaks have been reviewed in detail (11). Outbreaks documented since then are listed in Table 4. In 33 outbreaks during one year in Southampton and Chelmsford, England, 797 people suffered from shellfish-transmitted gastroenteritis. Epidemiologically, all of the outbreaks were traced to the consumption of cockles gathered from waters known to be polluted by sewage, and at a time when gastroenteritis was apparently widespread in a nearby area. In January 1980, 6 out of 13 persons (46%) in a small town in Florida, USA, became ill with Norwalk virus gastroenteritis after eating raw oysters. Norwalk virus infection was identified by a sensitive and specific serological radioimmunoassay.

An outbreak of hepatitis A occurred in 1980 among the members of the Japan Overseas Corporation Volunteers stationed in the Philippines. Seven out of 10 youths who ate raw oysters, but none out of five who did not, developed hepatitis. All these seven cases were positive for hepatitis A antibody and six of them were also positive for anti-HAV IgM 111 days after the infection.

During 1982, fourteen separate outbreaks of gastroenteritis associated with the consumption of raw clams occurred in the state of New York. Of the 150 persons affected, 7 subsequently developed hepatitis A some 21–37 days after eating the clams, and IgM antibody to hepatitis A virus appeared in their blood. Viral gastroenteritis in association with HAV is not known to have been reported following shellfish consumption. The clinical findings observed in several of the New York outbreaks are compatible with a viral etiology, such as the Norwalk virus: a short incubation period, abrupt onset of upper

Table 4. Outbreaks of shellfish-transmitted disease of documented viral etiology^a

Year	Source	Place	No. of cases	Virus
1973	Oysters	Houston, TX, USA	263	Hepatitis A
1976	Mussels	Australia	7	Hepatitis A
1978	Oysters	Australia	150	Norwalk
1978	Oysters	Australia	2000	Norwalk
1978	Mussels	England	41	Hepatitis A
1979	Oysters	Alabama, GA, USA	8	Hepatitis A
1976 to 1979	Cockles	England	797	Gastroenteritis
1980	Oysters	Florida, USA	6	Norwalk
1980	Oysters	Philippines	7	Hepatitis A
1982	Clams	New York, USA	150	Norwalk-like, Hepatitis A
1984	Cockles	Singapore	322	Hepatitis A

^a From Verber, J. L. In: *Shellfish-borne disease outbreaks*, Report from U.S. Food and Drug Administration, Shellfish Sanitation Branch. Northeast Technical Services Unit, Davisville, Rhode Island, June 1984.

and/or lower gastrointestinal illness, and brief duration (1–2 days). Absence of bacterial pathogens and the detection of virus-like particles in clams from one outbreak give further support to a viral etiology in several of these outbreaks.

An epidemic of 322 cases of serologically confirmed hepatitis A occurred between May and September 1983 in Singapore. The vehicle of transmission was traced to raw and partially cooked cockles imported from places with no sanitary control on the production (34).

HUMAN VIRUSES IN SEWAGE SLUDGES

Many viruses and bacteria sediment in the settling basins of primary treatment plants, in activated sludge settling tanks, and in the settling basins of other secondary and tertiary treatment processes. The Federal Water Pollution Control Act in the USA in 1972, which made at least secondary treatment of municipal sewage mandatory, resulted in huge increases in the amount of sewage sludge to be disposed. Management of sludge disposal became a major problem for many municipalities. Ocean dumping and land disposal were looked upon as the most economical methods for disposal of effluents and sludge. Since sewage sludge is a resource with a variety of uses, new emphasis was placed on understanding the fate of enteric viruses during sludge treatment and disposal.

Recovery of viruses from sludges

Since almost all of the enteric viruses contained in sewage sludges are believed to be solids-associated, the methods of virus recovery are directed towards the elution of viruses using beef extract, serum, detergent, or glycine followed by mechanical shaking or sonication and a reduction in volume of the eluate. An evaluation of the procedures for recovering enteroviruses from sludge has recently been published (12).

Quantity of viruses

Data on the quantity of viruses in raw and digested sludge are available from the studies of Berg & Berman (13) (Table 5). The numbers of viruses recovered from raw sludges

Table 5. Recovery of viruses from raw, mesophilically digested, and thermophilically digested sludges^{a, b}

Test No.	Viruses in sludge (PFU/100 ml)		
	Raw	Mesophilically digested	Thermophilically digested
1	380	30	<4
2	550	50	<3.1
3	1 530	—	<2.8
4	940	40	<2.7
5	2 780	280	3.3
6	1 550	250	<1.4
7	1 190	240	<1.7
8	1 810	100	1.7
9	11 600	410	5.0
10	2 470	200	5.7
11	1 500	360	16.7

^a Sludges consisted of one-third activated sludge and two-thirds raw primary sludge digested for about 20 days at 35 °C (mesophilic sludge) or at 50 °C (thermophilic sludge).

^b From Berg, G. & Berman, D. *Applied and environmental microbiology*, 39: 361–368 (1980).

ranged from 380 to 11 600 PFU/100 ml while mesophilic sludge (digested for about 20 days at 35 °C) contained viruses from 30 to 410 PFU and thermophilic sludge (digested at 50 °C) between <1.4 and 16.7 PFU/100 ml.

Fate of viruses during sewage sludge treatment

Heat treatment. The US Environmental Protection Agency's guidelines stipulated a temperature of 70 °C for 30 minutes as the minimum for pasteurization of sludge. Although enteric viruses are expected to be inactivated at this temperature, pasteurization of sludge is costly and energy-intensive. Studies by Ward et al. (14) demonstrated that enterovirus inactivation at lower temperatures (43 °C and 51 °C) was possible under certain conditions. These investigators detected an organic compound (an ionic detergent) in raw sludge which protected poliovirus. During anaerobic digestion of sludge, ammonia is formed and addition of the supernatant containing ammonia from the digested sludge to raw sludge solids, inactivated 99.99% of poliovirus in 5 minutes at 51 °C. By increasing the pH of the sludge from 8.4 to 9.0, the concentration of ammonia increases and under these alkaline conditions, "moderate" heat treatment of sludge was sufficient to inactivate the enteroviruses.

Anaerobic digestion. Anaerobic digestion is the most common sludge treatment in which microbes oxidize organic materials under anaerobic conditions and generate methane which can be used as fuel. Heat-dried sludges are used as fertilizers on crops and serve as excellent soil conditioners. In laboratory experiments, poliovirus 1 survived for more than 30 days when the virus was embedded inside sludge flocs prior to anaerobic digestion. Mesophilic digestion (35 °C) of raw sludge is not reliable for the inactivation of viruses, although the virus levels were, on average, reduced by about 80%, and following 45 days storage there was another 50% reduction. Thermophilic digestion (50 °C) had a greater virucidal effect than mesophilic digestion (15).

Sludge composting. Dewatered sludge is mixed with wood chips or leaves and allowed to decompose for about three weeks in summer and for more than one month in winter. Aerobic conditions are maintained either by pumping air into the compost pile or by regularly turning the pile. Temperatures between 50 °C and 70 °C are developed inside the compost pile. Studies on inactivation of poliovirus 1 in raw and composted sludge revealed that poliovirus was more heat-stable in raw than in composted sludge. This was attributed to degradation of ionic detergents which are associated with an ability to protect poliovirus from thermal inactivation (16).

Enteroviruses are known for their resistance to heat up to temperatures as high as 50 °C. This stability can be enhanced by the addition of MgCl_2 in molar concentrations to the incubating medium (17). Hepatitis A virus has been shown to survive heating to 60 °C for as long as 12 hours (18). More recently, the stabilities of HAV and of poliovirus type 2 were compared, as shown in Table 6, under controlled, identical conditions of pH, temperature, and salt concentration (19). Both viruses retained their infectivity when exposed to pH 3 to pH 10 at 20 °C for 30 minutes. Heating of poliovirus type 2 at 60 °C for 10 minutes led to an apparent complete loss of viral infectivity. Addition of 1 mol/l MgCl_2 allowed the virus to survive but with a marked decrease in titre. HAV, on the contrary, showed only a tenfold reduction in titre after heating to 65 °C for 10 minutes. In the presence of 1 mol/l MgCl_2 , HAV infectivity was effectively preserved, even at 80 °C.

Mechanisms of virus inactivation in anaerobically digested sludge were studied by Ward and colleagues. The virucidal agent in digested sludge was determined to be the uncharged form of ammonia (NH_3) and the site of action was the viral genome (20). Ammonia is believed to activate a nuclease within the virion or pass through hydrophobic regions of

Table 6. Effect of temperature, MgCl_2 , and pH on the infectivity of HAV and poliovirus type 2^a

Treatment ^b	HAV $\text{TCD}_{50}(\log_{10})$	Poliovirus $\text{TCD}_{50}(\log_{10})$
At pH 7:		
20 °C	5.6	8.8
60 °C	5.3	< 1.0
65 °C	4.3	< 1.0
80 °C	1.3	NT ^c
At pH 7 + 1 mol/l MgCl_2 :		
20 °C	5.8	8.8
60 °C	5.0	4.8
65 °C	5.6	2.8
80 °C	4.3	1.7
At 20 °C:		
pH 3	4.9	9.3
pH 4	5.0	9.3
pH 5	5.6	9.6
pH 7	4.9	9.3
pH 9	5.3	9.3
pH 10	6.0	9.8
pH 11	6.0	NT ^c

^a From Siegl, G. et al. *Intervirology*, 22: 218–226 (1984).

^b For heat treatment, the viruses were incubated for 10 min; for pH treatment, the incubation was extended to 30 min.

^c NT = not tested.

the capsid only in a non-ionized state, thus raising the virion internal pH sufficiently to permit alkaline hydrolysis of the viral genome.

Thermal inactivation of poliovirus at high temperatures occurs by an alteration of antigenicity and is accompanied by the release of one of four capsid proteins, VP4. Viral RNA, when released intact, is still infectious. At low temperatures, virus inactivation is attributed to a breakdown of viral nucleic acid within the antigenically unaltered viral capsids, and the RNA recovered was degraded and non-infectious.

During heat inactivation of HAV, destabilization of the virion results in the release of the genome and gives rise to the appearance of several RNA-free, probably “empty” capsid structures. Whether this process is preceded by the loss of one or more structural polypeptides from the viral capsid, as has been reported for poliovirus, is not yet known. It is also not known whether the loss of physical integrity of the virion is associated with a change in antigenicity (19).

The impact of sewage sludge disposal on the distribution of human enteric viruses at Philadelphia and New York Bight dumpsites was studied from 1980 to 1983 (23); 213 samples of sediment and 36 samples of water were examined. Human enteric viruses were isolated from 26 (12%) of the sediment samples and one (3%) water sample. Viruses were isolated from bottom-sludge sediments 64 km offshore and 17 months after cessation of sludge dumping. These results indicate that ocean sediments prolong virus survival and act as a reservoir of human pathogenic viruses following sludge disposal.

HUMAN VIRUSES IN SOILS

Urbanization and the resulting high-density population centres are exerting an increasing demand for technology to manage municipal wastes safely, effectively, economically, and within the acceptable limits of environmental tolerances. A need to reuse water,

as well as other resources contained in wastes, has led to intense interest in land application. At the present time, more than 700 cities in the USA are disposing a portion, or all, of their wastewater effluents and sludges in some land-based system, such as forests, golf courses, pastures and agricultural soils used for animal crop production. Important considerations favouring land disposal of wastes include (1) an increased awareness of the hazards associated with sewage pollution of surface waters, (2) the increasing demand on present sources of potable water, (3) the realization that valuable nutrients must be recycled, and (4) the need to recondition depleted soils in order to meet ever-increasing demands for human and animal crop production.

The disposal of wastewater and sludge by direct application to land should not be carried out without first considering the following potential health hazards: (i) direct virus infections of farm workers and their contacts, (ii) virus contamination of crops used for human consumption, (iii) virus contamination of drinking-water sources as a result of surface runoff or infiltration into ground water, and (iv) virus dissemination by aerosols during wastewater sprinkler irrigation with consequent risks of respiratory infection among farm workers, residents of adjacent areas, or travellers in the vicinity.

Techniques for the extraction, concentration, and enumeration of naturally occurring human viruses from soils are only marginally efficient for a few enteroviruses and, at the time of writing, totally inadequate for detecting hepatitis A virus, Norwalk virus, and human rotaviruses. Consequently, the virus recoveries reported in field studies should be considered as only partially quantitative. The finding of any enteric virus should be an indication that many other types may be present. The method of isolating virus from soil has been described (21).

Occurrence and distribution of viruses in soils

Viruses have been isolated from soils receiving domestic wastewater. Three different soil types containing 81%, 41%, and 7.6% sand were examined for their ability to adsorb coliphage f_2 from septic tank effluent. The great majority of viruses were detected within the first 15 cm of soil below the effluent application lines. Several isolates were found at a depth of 85 cm and single isolations at 100–120 cm. Hurst et al. (22) recovered 91% of seeded poliovirus from the top 2.5 cm of soil. The remaining 5.6% and 3.4% were isolated at 2.5–10 and 10–25 cm depths, respectively.

The studies of Landry et al (23) also indicated that 77% of seeded poliovirus in soil cores were detected in the top 5 cm of soil, 11% at 5–10 cm depths, 8% from the next 15 cm, and only 4% from the lower 50 cm of soil. All these studies indicate that most of the viruses are retained initially in the upper soil layers.

Adsorption, persistence and desorption of viruses in soils

Factors that influence the entry, survival, and migration of viruses in ground water are depicted in Fig. 3 and are discussed below.

Adsorption. Virus removal by soil is largely caused by adsorption which is controlled by a number of factors including composition of soil, ionic strength, pH, and flow rate of the water. Adsorption of 28 different animal and bacterial viruses to nine different soil types, using a batch adsorption procedure, indicated that poliovirus was adsorbed efficiently in the majority of test soils. Reference strains of coxsackieviruses (B1 through B6) were adsorbed readily in soils, whereas natural isolates of coxsackievirus B4 were adsorbed poorly (24). A great deal of variability was also observed in the adsorption of different strains of echovirus 1, indicating to some extent that virus adsorption to soils is strain-dependent. Adsorption to soil prolongs virus infectivity.

Fig. 3. Factors influencing the entry, survival and migration of viruses in ground water. Soils rich in clay minerals and percolating fluids with high ionic composition enhance virus retention; high levels of moisture and water in the soil promote virus survival while increased microbial activity tends to inactivate the viruses. Higher percentage of soil saturation with water and low ionic composition of soil fluids (like rain water) promote virus desorption from soil and help the movement of virus in ground water.

(Adapted from Melnick, J. L. & Gerba, C. P. *Public health reviews*, 9: 185-213 (1980))

Survival. During the past two decades many reports have described the persistence of enteroviruses in soil environments (25, 26). Poliovirus has been detected in soil irrigated with virus-seeded sewage sludge and effluent after 96 days in winter and 11 days in summer, and on the surface of mature vegetables 23 days after irrigation had ceased (27).

Desorption. Virus adsorption to soil is not an irreversible process and, under appropriate conditions, the viruses desorb and become entrained with percolating fluids. Some of the factors are presented below.

(i) **Concentration of ions.** The ion concentration or conductivity of percolating fluids appears to play a major role in virus desorption from soils. It has been reported that when soil columns containing adsorbed coliphage T7 were rinsed with deionized water, a number of phages apparently desorbed and were recovered in column effluents. Based on these laboratory results, the authors suggested that natural rainfall with low ionic concentration may act in the same manner, thus triggering virus migration at land application sites. This mechanism was, in fact, proposed by Wellings et al. (28) to explain the presence of enteric viruses in ground water beneath a spray irrigation system following a period of heavy rainfall (71 cm). Lance et al. (29) reported that the immediate application of sewage effluent to columns flooded with deionized water prevented extensive virus

movement by increasing the ion concentration. The investigators suggested that this technique could be employed as a practical and effective method for the management of operating recharge basins. The effects of rainfall would be countered by simply flooding the affected basins with sewage effluent.

(ii) *Virus type.* The adsorption of viruses to soils is dependent upon the virus type, which in turn appears to be governed by an overall electronegativity of the virus particle. It follows then that virus desorption should be similarly type-dependent. Less than 2% of a vaccine strain of poliovirus 1 was eluted from soil cores while large numbers of the mutants of this strain were extensively eluted with rain water. Vaughn et al. (30) suggested that all polioviruses may elute poorly, since members of this group were the least encountered during a field study of virus-contaminated aquifers. Recent field studies indicated that 18% of poliovirus 1 had moved from the core surface to a depth of 15 cm while 0.1% of echovirus was retained near the surface of the core.

(iii) *Other factors.* A number of other factors may also influence the desorption of viruses from soil. High pH fluids appear to create strong electrostatic repulsive forces between negatively charged soil and virus particles causing virus desorption. Since wastewater effluents are typically acidic in nature, the alkaline-influenced desorption process would not be expected to play a role in virus movements in environmental systems, except under unusual circumstances.

After desorption, several characteristics of the soil may contribute to virus movement to the ground water. Prominent among them are: soils riddled with cracks or fissures, depth of the soil profile, and degree of soil saturation. Noonan & McNabb (31) reported that coliphage ØX174 under saturated soil conditions moved laterally over distances of 900 m in a relatively short period of time, covering about 350 m/day.

HUMAN VIRUSES IN GROUND WATER

The application of wastewater and sludge, whether for agricultural irrigation or as a method of treatment and disposal, poses the possible risk of virus contamination of ground water. Over half of the waterborne disease outbreaks reported in the USA each year are caused by contaminated ground water.

Wastewater application rates are one of the critical factors in the possible penetration of viruses into ground water. While normal rates of water application for agricultural irrigation are about 1 m³ of water/1 m² of land/year, hydraulic loading rates for effluent disposal on land can be as high as 100 m³/m²/year. Virus removal declines with higher rates of loading. Exceptions exist in which a virus removal of at least 99.9% has been achieved despite application rates averaging 90 m³/m²/year of effluent to basins of loamy sand. No viruses were detected in wells 6 m deep and 6 m from the edge of the infiltration basin (32). On the other hand, Wellings et al. (28) isolated viruses from ground water after spray irrigation of the secondary sewage effluent onto a sandy soil. The viruses had survived chlorination, sunlight, spraying, and percolation through 3–6 m of the sandy soil; furthermore, after a period of heavy rain, a burst of viruses was detected in samples that had previously been negative. These studies demonstrate that soil type, rainfall, and other factors can affect viral movements into ground water, and that the viruses are capable of surviving long periods which, when combined with the ability to move long distances laterally, could lead to wide dispersal through an aquifer. Leachates from septic tanks can contaminate well waters. Wellings and coworkers (28) reported the isolation of an echovirus 22/23 complex in 100-gallon (379-litre) samples from a well

(12.2 m deep) during an outbreak of gastrointestinal illness at a migrant labour camp in Florida. The well was located 30.5 m from a solid-waste field, and was in the middle of an area bordered by septic tanks. Echovirus was isolated from sewage, from potable well water containing 0.4–0.6 mg/litre residual chlorine, and from stools collected from individuals living in the camp. Six weeks later, 15 cases of hepatitis A occurred in the camp. The virus was probably present in well water because of contamination from the septic tanks and existed in chlorinated water in the absence of bacterial contamination. Even though this level of treatment reduced the bacterial counts to undetectable levels, the virus survived. This raises concern about the efficacy of chlorine disinfection of contaminated ground water, based on currently accepted standards.

In the summer of 1980, a large outbreak of suspected viral gastroenteritis involved approximately 79% of 10 000 persons living in Georgetown, Texas (33). During the outbreak coxsackieviruses B2 and B3 were isolated from wells serving the city's water utility. Coxsackievirus B3 was also isolated from one sample of chlorinated tap water. An outbreak of 36 cases of hepatitis A followed 4 weeks after the gastroenteritis outbreak, in comparison with an expected incidence in this area of 0–2 cases per month. Hepatitis A antigen was identified both in the community's sewage and in one of the wells serving the city's supply. The viral antigen was detected in the water supply one month prior to the outbreak.

The Norwalk virus cannot yet be grown in tissue culture and no methods exist for its isolation from water. However, it has been implicated by serological methods in several outbreaks of gastroenteritis traced to contaminated well water.

CONCLUSIONS

(1) Viruses of public health significance are abundantly present in wastewater, and can be found even after secondary biological treatment and chlorine disinfection.

(2) Viruses are concentrated in wastewater sludges which may be ultimately used on land as fertilizers and soil conditioners.

(3) Increasing numbers of communities around the world are turning to land application of wastewater and sludges. Soils vary greatly in their capacity to adsorb viruses, and thus, to preclude ground-water contamination.

(4) Enteric viruses can remain viable for months in water and probably longer when associated with solids.

(5) Dissemination of solids-associated infectious viruses from polluted coastal estuarine waters to non-polluted recreational or shellfish-growing water may occur.

(6) Currently available methods for the extraction and concentration of viruses from sediments, sludges, and soils fail to detect the hepatitis A virus, Norwalk virus, or rotaviruses. Thus, there is a need to develop sensitive and quantitative methods for detecting these and other viruses of importance to human health.

(7) Better capabilities of virus detection coupled with efficient management practices in the disposal of wastewater, sludges, and sediments are needed to define the problem and avoid potential health hazards.

REFERENCES

1. MELNICK, J. L. *American journal of hygiene*, **45**: 240–253 (1947).
2. BERG, G. *Monographs in virology*, **15**: 17–29 (1984).

3. RAO, V. C. ET AL. *Indian Association for Water Pollution Control, Technical Volume 5*: 1-16 (1978).
4. BURAS, N. *Water research*, 10: 295-298 (1976).
5. NUPEN, E. M. *Water research*, 4: 661-667 (1970).
6. STAGG, C. H. ET AL. *Progress in water technology*, 10: 381-387 (1978).
7. RAO, V. C. ET AL. *Applied and environmental microbiology*, 48: 404-409 (1984).
8. WAITE, D. A. & SOBSEY, M. D. *Applied and environmental microbiology*, 46: 379-385 (1983).
9. SMITH, E. M. ET AL. *Applied and environmental microbiology*, 35: 685-689 (1978).
10. CABELLI, V. J. ET AL. *American journal of epidemiology*, 115: 606-616 (1982).
11. GERBA, C. P. & GOYAL, S. M. *Journal of food protection*, 41: 743-754 (1978).
12. GOYAL, S. M. ET AL. *Applied and environmental microbiology*, 48: 531-538 (1984).
13. BERG, G. & BERMAN, D. *Applied and environmental microbiology*, 39: 361-368 (1980).
14. WARD, R. L. ET AL. *Applied and environmental microbiology*, 33: 339-346 (1976).
15. MOORE, B. E. ET AL. In: *Sludge management disposal and utilization, Proceedings of the Third National Conference on Sludge Management Disposal and Utilization*. Rockville, MD, Information Transfer Inc., 1977, pp. 108-112.
16. WARD, R. L. & ASHLEY, C. S. *Applied and environmental microbiology*, 36: 898-905 (1978).
17. WALLIS, C. ET AL. *Virology*, 26: 694-699 (1965).
18. FRÖSNER, G. G. *Öffentliche Gesundheitswesen*, 44: 370-373 (1982).
19. SIEGL, G. ET AL. *Intervirology*, 22: 218-226 (1984).
20. WARD, R. L. *Monographs in virology*, 15: 175-183 (1984).
21. BITTON, G. ET AL. *Canadian journal of microbiology*, 25: 874-880 (1979).
22. HURST, C. J. ET AL. *Applied and environmental microbiology*, 40: 192-200 (1980).
23. LANDRY, E. F. ET AL. *Applied and environmental microbiology*, 40: 1032-1038 (1980).
24. GOYAL, S. M. & GERBA, C. P. *Applied and environmental microbiology*, 39: 241-247 (1979).
25. DUBOISE, S. M. ET AL. *Critical reviews in microbiology*, 7: 245-259 (1979).
26. GERBA, C. P. ET AL. *Journal of the Irrigation Drainage Division of the American Society of Civil Engineers*, 101: 157-174 (1975).
27. TIERNEY, J. T. ET AL. *Applied and environmental microbiology*, 33: 109-113 (1977).
28. WELLINGS, F. M. ET AL. In: *Virus survival in water and wastewater systems*, Austin, University of Texas Press, 1974, pp. 253-260.
29. LANCE, J. C. ET AL. *Applied and environmental microbiology*, 32: 520-526 (1976).
30. VAUGHN, J. M. ET AL. *Applied and environmental microbiology*, 36: 47-51 (1978).
31. NOONAN, M. J. & McNABB, J. F. In: *The quality and movement of ground water in alluvial aquifers*, Christ Church, New Zealand, Lincoln College, 1979, pp. 195-201.
32. GILBERT, R. G. ET AL. *Science*, 192: 1004-1005 (1976).
33. HEJHAL, T. W. ET AL. *Journal of American Water Works Association*, 74: 318-321 (1982).
34. GOH, K. T. ET AL. *Bulletin of the World Health Organization*, 62: 893-897 (1984).