

Albert Bosch

Department of Microbiology,
University of Barcelona, Spain

Received 19 June 1998
Accepted 27 July 1998

Correspondence to: Albert Bosch, Department
of Microbiology, University of Barcelona,
Diagonal, 645, 08028 Barcelona, Spain.
Tel.: +34-934021485. Fax: +34-934110592.
E-mail: albert@porthos.bio.ub.es

Human enteric viruses in the water environment: a minireview

Summary Water virology started around half a century ago, with scientists attempting to detect poliovirus in water samples. Since that time, other enteric viruses responsible for gastroenteritis and hepatitis, among a great variety of virus strains, have replaced enteroviruses as the main target for detection in the water environment. Technical molecular developments, polymerase-chain reaction (PCR) amplification being the method of choice, enable the detection of fastidious health-significant viruses. However, shortcomings of molecular procedures include their potential incompatibility with concentration methods, indispensable to reduce the water sample volume to assay for viruses, and the inability to discern between infectious and non infectious material. On the other hand, these procedures are restrained to sophisticated laboratories and detection of alternative indicator organisms has been proposed. Bacterial indicators fail to give a reliable clue of the virological quality of water. Selected bacteriophage groups appear as a better choice for their use as virus indicators.

Key words Enteric viruses · Rotaviruses · Hepatitis · Gastroenteritis · Water environment

Introduction

As a scientific discipline, water virology was born after a large hepatitis outbreak was declared in New Delhi between December 1955 and January 1956. The origin of the outbreak was the contamination by sewage, from one to six weeks prior to the epidemic, of Jumna river, the source of water for the treatment plant. Alum and chlorine treatment prevented bacterial infections, but 30,000 cases of hepatitis occurred among the population. Water virology, actually environmental virology, began with efforts to detect poliovirus in water around half a century ago.

The characters

Present in sewage contaminated waters are over 100 virus species which cause a wide variety of illnesses in man. These include hepatitis, gastroenteritis, meningitis, fever, rash, conjunctivitis, and maybe diabetes (Table 1). However, only a few of these viral pathogens have been shown epidemiologically to be waterborne transmitted [13]. In 1979 it was estimated that between 5 and 18 million people die every year from gastroenteritis, and rotaviruses alone are responsible for over 1 million children dying from diarrhea [17]. Additionally, the Mediterranean region is endemic for hepatitis A, and poliomyelitis has not yet been eradicated, although it is one of

the objectives pursued by the WHO for year 2000. Adenovirus, astrovirus, Norwalk and other small round structured viruses (SRSV), as well as hepatitis E, are other health significant viruses that may be acquired by ingestion of contaminated water or shellfish. The mortality rates reported by different sources for some of these viruses are shown in Table 2.

Table 1 Human enteric viruses that may be waterborne transmitted

Genus	Popular name	Disease caused
<i>Enterovirus</i>	Poliovirus	Paralysis, meningitis, fever
	Coxsackievirus, A, B	Herpangina, meningitis, fever, respiratory disease, hand-foot-and-mouth disease, myocarditis, heart anomalies, rash, pleurodynia, diabetes?
	Echovirus	Meningitis, fever, respiratory disease, rash, gastroenteritis
<i>Hepatovirus</i>	Hepatitis A	Hepatitis
<i>Reovirus</i>	Human reovirus	Unknown
<i>Rotavirus</i>	Human rotavirus	Gastroenteritis
<i>Mastadenovirus</i>	Human adenovirus	Gastroenteritis, respiratory disease, conjunctivitis
<i>Calicivirus</i>	Human calicivirus	Gastroenteritis
	Norwalk virus	Gastroenteritis, fever
	SRSV	Gastroenteritis
	Hepatitis E	Hepatitis
<i>Astrovirus</i>	Human astrovirus	Gastroenteritis
<i>Parvovirus</i>	Human parvovirus	Gastroenteritis
<i>Coronavirus</i>	Human coronavirus	Gastroenteritis, respiratory disease
<i>Torovirus</i>	Human torovirus	Gastroenteritis

Table 2 Mortality rates for human enteric viruses in developed countries (data from references [6] and [12])

Virus	Mortality rate (%)
Poliovirus 1	0.90
Coxsackievirus A	0.12–0.50
Coxsackievirus B	0.59–0.94
Echovirus	0.27–0.29
Hepatitis A	0.60
Rotavirus	0.01–0.12
Norwalk	0.0001
Adenovirus	0.01

The problem

Human enteric viruses enter the water environment through the discharge of sewage contaminated water. Viruses are shed in extremely high numbers in the feces of infected individuals: patients suffering from gastroenteritis or hepatitis may excrete from 10^5 to 10^{11} virus particles per gram of stool [17]. Sewage sludge is a complex mixture of solids of biological and mineral origin that are removed from wastewater in sewage treatment plants. Sludge is a by-product of physical (primary treatment), biological (activated sludge, trickling filters) and physicochemical (chemical precipitation with lime, ferric chloride or alum) treatment of wastewater. The type of treatment will determine the concentration of pathogens and the relative risk of disposal. Viruses are present in high numbers in raw wastewater and current water treatment practices fail to ensure the complete removal of viral pathogens [35]; consequently, viruses become environmental pollutants. Solid-associated viruses in wastewater effluents are discharged into aquatic environments and accumulate in the sediments where they persist longer than in the water column [41]. As a matter of fact, sediments act as a reservoir from which viruses are resuspended in the water column by several natural or artificial phenomena [11, 36, 39].

The fate of microbial enteric pathogens may take many potential routes in the water environment. Fig. 1 illustrates the possible routes of transmission of enteric viruses. Humans are exposed to enteric viruses through various routes: shellfish grown in contaminated waters, food crops grown in land irrigated with wastewater and/or fertilized with sewage, sewage-polluted recreational waters and contaminated drinking water. Usually, waterborne infections are acquired through the ingestion of contaminated water or shellfish. Besides, surface and ground waters are employed for public consumption. In a study of waterborne disease outbreaks reported between 1946 and 1980 [31], water system deficiencies that caused or contributed to these outbreaks were categorized under five major headings: (i) use of contaminated, untreated surface water; (ii) use of contaminated, untreated

groundwater; (iii) inadequate or interrupted treatment; (iv) distribution network problems; and (v) miscellaneous. More than 80% of the outbreaks were associated with deficiencies in treatment and distribution of water. On the other hand, shellfish grown and harvested in virus contaminated waters are a well documented source of gastroenteritis or hepatitis [14, 37]. Shellfish, being filter feeders, tend to concentrate viruses and bacteria in their edible tissues, and concentrations of these microorganisms in shellfish may be expected to be much higher than in the surrounding water [1]. Enteric viruses may potentially also be transmitted by recreational activities in polluted waters [44].

The risk of waterborne infection

Some viral infections are spread by means of continual low-level transmission through the environment. The nature of most enteric virus diseases is such that they elude epidemiological studies. Many viruses cause inapparent or silent infections that go unrecognized until secondary person-to-person spread finally leads to overt disease in hardly traceable pockets of the population. The exact risk of illness from enteric viruses in water after exposure is hard to quantitate. Important in any risk assessment is the level of concentration of contaminant which is required to cause a health effect. Ideally, maximum contaminant levels for potentially harmful substances would be established on firm epidemiological evidence, where cause and effect can be clearly quantitated to determine a minimum- or no-risk level. However, while epidemiology is a valuable tool for determining patterns of risk and establishing statistically significant associations with risk agents, cause and effect cannot be easily demonstrated. Exacting data on minimum infection dose for humans is generally unfeasible because of the extreme cost, ethical restrictions of human experimentation, and uncertainty in extrapolating dose-response curves to low exposure levels. However, a formal risk assessment of microbial exposure may be conducted under the standard framework employed for chemical risk assessment. This consists of three steps: dose-response assessment, exposure assessment and risk characterization. The microbial risk quantification may be performed using point estimation methods, such as maximum likelihood estimates of dose-response and maximum exposed individual, or estimates for the entire population based on the frequency distribution of the exposure [20, 22, 38].

Fortunately, not everyone who becomes infected with enteric viruses will become clinically ill. Asymptomatic infections are particularly common among some enteric viruses. The development of clinical illness depends on numerous factors including the immune status and age of the host, type, strain and virulence of the microorganism, and route of infection. For instance, for hepatitis A virus (HAV)

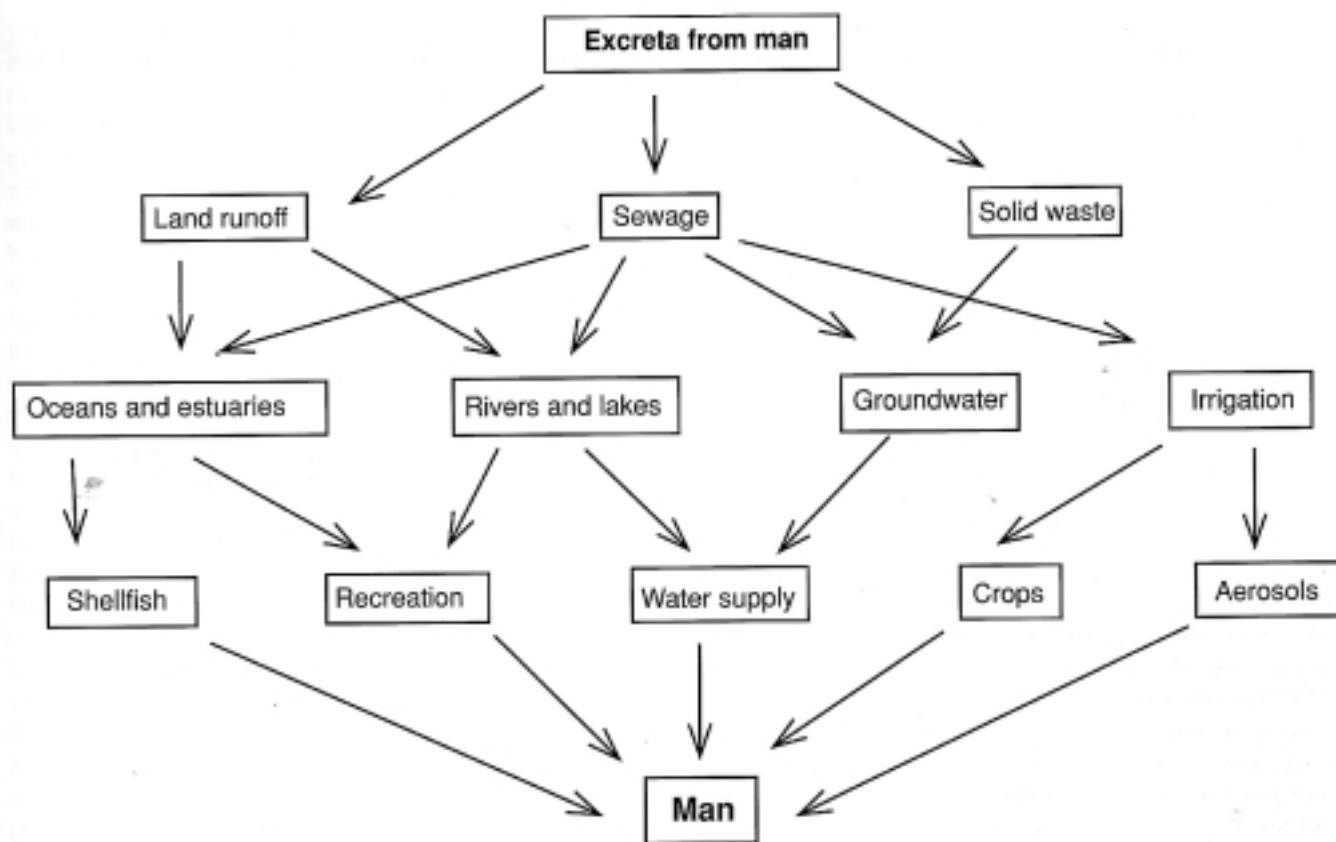


Fig. 1 Routes of enteric virus transmission (adapted from reference [35])

the percentage of individuals with clinically observed illness is low for children but increases greatly with age. In contrast, the frequency of clinical symptoms for group A rotavirus infections is greatest in childhood and lowest in adulthood. While the frequency of clinical hepatitis is estimated at 75%, during waterborne outbreaks it has been observed as high as 97% [30]. Table 3 shows the probability of infection for some waterborne enteric viruses. Mortality rates are also affected by many of the same factors which determine the likelihood of development of clinical illness. The risk of infection is 10 to 10,000 times less for bacteria than for viruses and protozoa at a similar level of exposure [22].

Table 3 Probability of infection from exposure to 1 virus and the dose required for 1% chance of infection. Models are based on dose-response curves developed from human feeding studies and assumed consumption of 2 liters of water per day (adapted from references [20, 22, 38])

Virus	Probability of infection from exposure to 1 virus	Dose for 1% chance of infection
Poliovirus 1	0.0149	0.67
Poliovirus 2	0.0310	0.32
Echovirus 12	0.0170	0.59
Rotavirus	0.3100	0.03

The error

One critical question in environmental virology is whether or not these viruses can survive long enough and in concentrations high enough to cause disease in individuals who are in contact with polluted recreational water, or who consume contaminated water or seafood. The survival of enteric viruses in the environment has been reviewed elsewhere [3, 5, 8]. As a rule, viruses persist longer than enteric bacteria. It is then completely unsafe to rely on bacteriological standards to assess the virological quality of any kind of water. Reports exist on waterborne outbreaks related to potable water that met bacteriological standards [10, 24]. For instance, in an outbreak of infectious hepatitis which occurred among a military community, HAV, rotaviruses and enteroviruses were detected in water samples that were consistently free of indicator bacteria [10]. These same samples showed free and total chlorine levels that were adequate to ensure a proper elimination of bacterial contaminants, but were unable to remove pathogenic viruses. This kind of studies support the recommendation of the monitoring of viral contamination, and the setting up of a

more powerful water treatment once viruses are detected in the water supply. The possibility nowadays to detect the presence of human enteric viruses in a water source should be a most valuable tool in the prevention of waterborne diseases. Unfortunately, in most waterborne outbreaks, samples are not assayed for the presence of human pathogenic viruses until after the outbreak. Consequently, no prophylactic measures may be taken among the population in order to decrease the severity of the potential outbreak.

The tools for the prevention of waterborne outbreaks

The basic steps of the virological analysis of water are sampling, concentration, decontamination/removal of inhibitors, and specific virus detection. Concentration of water samples is a critical step, since the viruses may be present in such low numbers that it is necessary to reduce the volume of the sample to be assayed to a few milliliters. A good concentration method should fulfil several criteria: it should be technically simple, fast, provide high virus recoveries, be adequate for a wide range of enteric viruses, provide a small volume of concentrate, and be inexpensive.

Table 4 shows a broad selection of currently available and widely employed procedures. Methods based on the adsorption of viruses from a large volume of water onto a suitable solid surface, from which they may be subsequently eluted into a much smaller volume, are recommended for their use with large volume samples. Details on virus concentration procedures have been published elsewhere [15, 16, 19, 42].

Table 4 Procedures for the concentration of viruses from large volumes of water

Adsorption-elution methods	
	Negatively charged filters
	Positively charged filters
	Glass powder
	Glass fiber
	Aluminium hydroxide
Precipitation	
	Organic flocculation
	Ammonium sulfate
	Polyethyleneglycol hydroextraction
Ultrafiltration	

As Metcalf and collaborators pointed out in their excellent review [32], a new era in environmental virology began in the 1980s, with the introduction of several significant developments. These were: (i) the recognition of hepatitis E virus as an enteric virus capable of producing waterborne epidemics; (ii) the adaptation of strains of HAV to replicate in cell culture and the development of sensitive

assays for this virus; (iii) the development of methods for the concentration of HAV from water; (iv) the recognition of the involvement of HAV in waterborne epidemics; (v) the evidence, after seroepidemiological surveys of outbreaks of non bacterial gastroenteritis, that SRSV, including Norwalk virus, is an important etiological agent of diarrhea; (vi) the demonstration of the involvement of human rotavirus in infantile gastroenteritis; (vii) the recovery of new types of enteric adenoviruses, caliciviruses and astroviruses from children suffering from acute diarrhea; and (viii) recognition that outbreaks of hepatitis and gastroenteritis could be caused by the transmission of enteric viruses in the environment.

Health-significant viruses, which were previously unrecognizable because they replicate poorly or not at all in cell cultures, became detectable with the advent of nucleic acid based techniques. Environmental virologists employed hybridization assays which have been more recently replaced by polymerase chain reaction based procedures [9, 18, 28, 29, 33, 40].

However, not only viruses are concentrated: PCR-inhibitory substances are concentrated along with the viruses. Consequently, procedures must be subsequently performed for the removal of inhibitors from the virus concentrates if molecular procedures would be applied for virus detection. A great variety of procedures have been developed for the removal of inhibitors from the virus concentrates. These procedures are: dialysis, solvent extraction, proteinase treatments, gel or glass filtration, nucleic acid adsorption or precipitation, lyophilization, antigen-capture PCR, and cell culture passage [4, 7, 21, 26, 45]. The degree of virus detection effectiveness achieved by PCR is the result of two related factors: the efficiency of recovery of the concentration procedure from the water sample, and the degree of final purity of recovered viruses.

Molecular techniques fail however to discern between infectious and non-infectious particles, which may be of critical relevance in environmental virology. A long time pursued objective is the use of cell lines susceptible to support the propagation of a wide variety of enteric viruses, enabling the amplification of virus sequences in cell culture prior to detection by PCR, accomplishing the dual purpose of increasing the number of copies of target nucleic acid and of incorporating an infectivity assay as well [34]. Whenever possible, the use of a combined cell culture-PCR procedure utilizes the major advantages of the separate methodologies, while overcoming many of their disadvantages. Dilution of the sample by culture media, coupled by an increase in infectious virus numbers, provides a way to reduce the effect of toxic compounds on cell culture and inhibitory substances on PCR. At the same time, this technique indirectly increases the sample volume actually assayed by PCR, and consequently the chance for the rapid detection of infectious viruses.

The use of indicator microorganisms

From an epidemiological point of view, the most relevant viral pathogens found in water are the hepatitis A and E viruses, and the gastroenteritis viruses, which include rotaviruses, caliciviruses (with SRSV, notably the Norwalk-like viruses), astroviruses and enteric adenoviruses. Due to technical difficulties, tests for most of these viruses remain restricted to laboratories with sophisticated facilities and well-trained personnel. On the other hand, it is impracticable to monitor the presence of all viral pathogens. This led to the origin of the concept of indicator microorganism. Since we are concerned with viruses transmitted through the fecal-oral route, microorganisms present in the fecal microbiota were immediately proposed. However, reliance on bacterial model microorganisms does not guarantee water to be free from enteric viruses. A better choice is to look for alternative, more reliable, indicators for viruses. A good indicator should fulfil the following requirements: (i) should be associated with the source of the pathogen and should be absent in unpolluted areas, (ii) should occur in greater numbers than the pathogen, (iii) should not multiply out of the host, (iv) should be at least equally resistant to natural and artificial inactivation as the viral pathogen, (v) should be detectable by means of easy, rapid and inexpensive procedures, and (vi) should not be pathogenic. Obviously the "ideal" indication is provided by the viral pathogen itself; however, three bacteriophage groups appear as promising candidates: somatic coliphages, F-specific bacteriophages and *Bacteroides fragilis* bacteriophages [23, 25, 27]. The International Organization for Standardization (ISO) has elaborated procedures for detecting these bacteriophages in water (ISO 10705-1, ISO 10705-2, ISO 10705-4).

The vaccinal strain of poliovirus 1 has been extensively used as model strain. However, evidence shows that poliovirus does not provide an adequate indication of the behavior of human enteric viruses, such as hepatitis A virus or rotaviruses, in the environment under natural or disinfection conditions [2, 43].

Exhaustive studies are required to ascertain the validity of a candidate indicator in a given situation, i.e., drinking water, recreational water, shellfish-growing water, irrigation water, reclaimed wastewater, etc. In the end, we will probably give up our hopes of finding a "universal" indicator for viruses and resign ourselves to the use of different indicators, or actual cell-adapted laboratory strains of fastidious enteric viruses, for specific different purposes. This problem, however, remains a real challenge to water virologists.

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