Problems in Detecting Water-Borne Viruses by Rapid Methods

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Introduction

The viral etiology of outbreaks of water-borne disease is epidemiologically difficult to prove, and evidence is limited to a small fraction of the many types of human pathogenic viruses found in water (IAWPRC Study Group on Water Virology 1983). In spite of this, there is a growing concern with the virological quality of water. Classical bacterial indicators of sewage pollution have not always been adequate, and infectious virus has been found in water samples meeting coliform standards (Deetz et al. 1984)

Routine monitoring of water for the presence of viruses is not generally performed, largely due to the current state of the methodology, which is difficult, expensive, and time-consuming. Steps in the assay of water for viruses include obtaining a representative sample of water, concentration of the viruses from the water, detection of the viruses, and identification. This paper is concerned with the use of rapid methods in one of these stages, that of virus detection. A useful method for detecting water-borne viruses should warn of the presence of viruses before the water is consumed. Rapid methods of virus detection, if adaptable to the requirements of the water environment, would present an attractive approach to the water virologist.

Requirements of a Water-Borne Virus Detection Method

The water environment presents difficulties in virus detection which differ in several ways from the problems of clinical isolation. Although speed of detection is important, a number of other points should also be considered in the choice of an appropriate method for the detection of water-borne viruses.

In the water environment, human pathogenic viruses are present in low concentrations due to dilution, adsorption to solids, and extinction. A primary requirement for a virus detection method is sensitivity. The sampling of large volumes of water is required, and in recent years techniques for concentrating viruses from water have been developed and tested, and are undergoing further improvement (Melnick et al. 1984). However, even one infectious virus in hundreds of

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liters of drinking water is a cause for concern, and a virus detection method should be able to detect very small numbers of virus particles.

Pathogenic viruses enter water primarily through contamination by human or animal waste. Wastewater contains a variety of viruses which are present in the population and contribute in an additive way to the levels of pollution. A virus detection method should be specific in the detection of pathogens, yet be sensitive to a broad range of viruses. It is difficult to predict which viruses will be present in water at a given time, and variations may be hourly, seasonal, or annual. Since there is no single detection method for all water-borne viruses, a battery of tests would have to be used to cover the full complement of viruses. In practice, a single method is often used to detect a limited range of viruses. If a particular virus is of interest, then the choice of method is simplified to selecting the one which is most appropriate for the chosen virus.

The water environment contains contaminants, both chemical and biological, which may interfere with the virus detection method. This is particularly problematic with wastewater, which may contain high levels of toxic materials as well as large numbers of microorganisms. A detection method should be evaluated for various types of water to determine its usefulness with natural waters. In addition, the concentration of viruses from water may be accompanied by the concentration of interfering materials. A detection method should therefore be tested in conjunction with a concentration technique.

A virus detection method should differentiate between viable virus and viral remnants which are no longer infectious. Following treatment and disinfection, inactivated viral elements may remain in the water but no longer represent a health hazard.

Virus identification is desirable, but is not an absolute requirement. It is generally more important to know whether there are viruses in the water than to know which ones. However, it is often of interest to obtain a profile of the viral flora, and the detection method should provide for this option by directly identifying or enabling identification of the viruses.

Finally, a virus detection method for routine monitoring should be used frequently and be practicable for public health laboratories to perform. This is limited in part by cost, as well as simplicity of use. In order for the results of the virus assays to have validity, the method should be reliable and accurate.

If a method were found which met all of the above points, it would provide a practical approach to the reduction of water-borne disease. In reality, there is no such ideal method, and the choice depends of the aims of the user. For monitoring drinking water, the most sensitive and rapid method should be chosen. In this case, identification is of less importance than the knowledge that the water is contaminated by viable virus. For studies of sewage and the effects of treatment processes, the types and amounts of virus present are of interest. In this case, a less sensitive method may be acceptable, since the high virus concentrations in the wastewater are relatively easy to detect. Under certain circumstances, such as the investigation of a disease outbreak caused by a particular virus, water samples may be assayed for the suspected agent.

Water-Borne Viruses

Viruses which are water-borne share a number of characteristics. They are relatively stable in water and remain viable until they reach a new susceptible host. Most of the water-borne viruses are enteric and replicate in the intestinal tract of the host, although they are not necessarily restricted to that site.

The types of water-borne viruses are listed in Table 1. These include viruses which have been shown to cause water-borne outbreaks of disease (such as hepatitis A virus), and others which may have that potential. Potential water-borne viruses are those which are found or excreted into water, but have not yet been shown to transmit disease via water (such as reoviruses). In total, there are more than 120 types of human pathogenic viruses which may be found in the water environment. These viruses have been reviewed elsewhere (Banatvala 1981), and the following discussion emphasizes aspects of the viral groups relevant to their assay in water.

The enteroviruses are a group of 72 viruses containing single-stranded RNA, and are members of the picornavirus family. Although they frequently cause subclinical infections, they are also associated with more serious illnesses such as meningitis, paralysis, and hepatitis. Generally, antisera prepared against one type of enterovirus do not cross-react with the other types, and they are assigned to the group on the basis of morphology and composition.

Included in this group are the polioviruses, coxsackie B viruses, and echoviruses, which are relatively easily isolated and cultivated by tissue culture methods. Other members of this group are more problematic; for example, some types of coxsackie A viruses are difficult to cultivate in tissue culture. Hepatitis A virus, which has recently been designated enterovirus 72, is a virus of importance in the transmission of water-borne hepatitis. It is a virus which is cultivated with difficulty and has not been detected in water by tissue culture methods.

The enteroviruses as a group are frequently found in water, in part because of their abundance and in part because they are relatively easy to detect in cell

Table 1. Water-borne viruses

Virus group	Representative types	Group-specific antigen?
Enteroviruses	polioviruses coxsackie A viruses coxsackie B viruses echoviruses hepatitis A virus	
Reoviruses		Yes
Adenoviruses	enteric adenoviruses	Yes
Rotaviruses		Yes
Parvovirus-like	Norwalk agent	
Other fecal viruses	astroviruses coronaviruses caliciviruses	

cultures. This is particularly true of the polioviruses, which are common in areas where they are administered to the population in the form of attenuated live vaccines. The levels of specific enteroviruses in different water samples may vary widely (Morris and Sharp 1982), making it difficult to predict in advance which types will be present.

The reoviruses are a group of three virus types which contain double-stranded RNA. They replicate well in tissue culture and can be isolated directly from water. In some cases their abundance in water has been reported to exceed that of enteroviruses (Grabow and Nupen 1981). However, relative virus numbers are influenced by the tissue culture systems used in the specific assay (Sellwood and Dadswell 1981), and may not accurately reflect the absolute quantities of virus found in water.

The reoviruses share group-specific antigenic determinants so that an antiserum prepared against one should detect all three. Rapid immunoassays for these viruses are a feasible approach to their detection in water.

The adenoviruses are a group of DNA viruses which primarily cause upper respiratory tract infections, although they are also excreted into water. Recently new strains (40 and 41) of enteric adenoviruses associated with gastrointestinal disease which are difficult to cultivate in tissue culture have been found (Wigand et al. 1983). Although most work has indicated that adenoviruses are present in lower abundance in water than the enteroviruses or reoviruses (Irving and Smith 1981; Goddard and Sellwood 1982), this may be due to the inefficiency of the tissue culture methods used, which would not have detected the "fastidious" enteric adenoviruses. There is antigenic cross-reactivity among the adenoviruses, and a group-specific antibody might be a useful reagent in their detection in water.

The rotaviruses, which contain double-stranded RNA, are a major cause of infantile gastroenteritis. The human rotaviruses cross-react serologically and are currently typed into four subgroups (Wyatt et al. 1983). Although they have not been detected in water using standard tissue culture techniques, the recent application of rapid methods has allowed their detection, as will be described below.

The parvo-like viruses include the Norwalk agent, which has been shown to cause gastroenteritis and to be transmitted by water (Kaplan et al. 1982). These viruses have not yet been cultivated outside of a human host and have not yet been directly detected in water. Rapid methods may be the only useful ways to demonstrate their presence in the water environment.

Other viruses which are excreted into water may be transmitted through the environment; here all viruses are suspect. Some of the additional viruses listed in Table 1 may play a role in disease transmission via water, but their importance as water contaminants has yet to be investigated.

Although there are a large number of virus types which can be found in water, it may not be necessary to detect them all. Once their relative abundance and importance is better established, it may be possible to choose one or a few groups to indicate viral pollution and warn of possible contamination with other viruses as well.

Rapid Methods: Useful for Detecting Water-Borne Viruses?

Viruses may be detected by their effects on a host, whether an animal or cells in culture. These are considered to be standard methods of virus determination and detect replication, and tissue cultures are to be preferred over animals whenever possible. Rapid methods, which by definition detect viruses in a shorter time than the standard methods, usually detect components of the virus, such as the physical particle (electron microscopy) or its subunits (proteins by immunoassays and nucleic acid by hybridization).

The most common methods of virus isolation and enumeration in water are standard tissue culture techniques (cytopathic effect or plaque assay). However, not all of the water-borne viruses are detectable in cell culture, even when several lines from different sources are used. Water-borne viruses are not tissue culture adapted, making them more difficult to detect than laboratory strains. For those viruses which do grow well in tissue culture, such methods are sensitive to small numbers of viruses, and only viable viruses are detected. It is possible to choose a cell type sensitive to a broad range of viruses likely to be found in water, without having to preselect for specific viruses. In virus mixtures, however, more rapidly growing viruses may overgrow the slower viruses, preventing their detection. Tissue culture methods are well established in many laboratories, do not require expensive reagents, and are not technically difficult to perform. One major difficulty with tissue cultures is that they are often affected by toxic components in water samples, which could prevent detection of any viruses present. In addition, tissue culture techniques which can take several weeks to detect viruses may be too slow to enable the prevention of virus spread by the water route.

There is a continuing search to find more rapid methods for virus detection which are sensitive enough to be useful for water-borne viruses. The advantages and disadvantages of some general approaches to the rapid detection of viruses as applied to the water environment are discussed below. These methods are able to detect the presence of virus from within a few hours to a day. Although some require sophisticated equipment, once instituted they are not technically difficult to perform.

Electron Microscopy

Electron microscopy (EM) is a way to directly visualize virus particles in a sample. Viruses which have a distinctive morphology can be identified by this method, and all viruses present in sufficient concentrations in the sample should be detectable. A further refinement, the addition of antibodies to aggregate specific viruses (immunoelectron microscopy or IEM) allows more precise identification of the viruses, but limits the range of viruses detected. This would increase the sensitivity of the assay for the specific viruses chosen. The major drawbacks of EM and IEM are that they do not differentiate between viable and nonviable particles and are not sensitive to small numbers of virus particles (Elkana and Guttman-Bass 1983). Another problem is that environmental samples may contain large amounts of nonviral material which may obscure the presence of viruses. In ad-

dition, particles which resemble viruses may be falsely identified as human pathogens, particularly with viruses without distinguishing characteristics (apart from size and shape) such as the enteroviruses.

Immunoassays

Immunoassays utilize antibodies prepared against specific viruses to detect the presence of viral proteins in a sample. A preliminary selection must be made as to which viruses to detect, which in virus monitoring is a major drawback. A method for detecting water-borne viruses should be as general as possible, since the composition of the viral flora in water at any given time is not predictable. However, these approaches are useful for certain viruses difficult to detect by tissue culture methods, or for groups of viruses sharing a common antigen.

Immunoassays in common use include radioimmunoassays (RIA) and enzyme-linked immunsorbent assays (ELISA), in which the antigen is detected by antibodies labelled with a radioactive or enzymatic marker. These assays are rapid and easily automated, but are not as sensitive as tissue culture techniques. They are specific for viruses with antigenic components which can be detected by the antibody used, and are thus limited in range. Such immunoassays might prove to be useful for the detection of viruses with group-specific determinants in water. These immunoassays are probably less sensitive to interference by biological contaminants than tissue culture methods, but may be affected by chemical contaminants in the samples. Unless used in combination with a growth enrichment step, they do not differentiate between viable and noninfectious particles. One advantage of the ELISA is its portability; it can also be adapted to on-site virus detection more easily than the other methods.

Immunofluorescent (IF) methods, in which viral antigens are detected in infected cells by antibody tagged with a fluorescent probe, have a number of advantages over RIA and ELISA, although results may take longer to obtain (generally within 24 h). IF methods can be as sensitive as tissue culture techniques and can theoretically detect as little as one infectious virus unit. Viable virus is detected by IF more rapidly than in standard tissue culture assays. As with the other immunoassays, a specific antibody must be chosen in advance. It is possible, however, to combine antibodies to increase the range of virus detection (N Guttman-Bass and Y Tchorsh, unpublished results, 1983). There is a shorter time for microbial growth and contamination to interfere with the assay than with standard tissue culture methods, but since the assay uses cells, it is probably more sensitive to interference than the assays using only chemical reagents. The assay is limited not only by the antibodies selected, but will also only detect those viruses which produce antigen in the particular host cell used.

Nucleic Acid Hybridization

Nucleic acid sequences specific to viruses can be detected by hybridization to a labelled probe complementary to viral sequences. This method has not yet been

used to detect viruses in water, but is discussed here as a possible direction for future investigation. The low virus concentrations in water would probably not allow the direct detection of viral sequences, but enrichment could be attained by growth in cell culture. Only partial growth would be necessary, to the stage where replication of the viral genome or accumulation of viral RNA would reach detectable concentrations. For virus groups with shared sequences, a probe complementary to that region could be used to detect the virus group. This assay would thus measure viable virus and might prove to be highly sensitive.

Current Use of Rapid Methods to Detect Water-Borne Viruses

The application of rapid methods to the detection of water-borne viruses is expanding as reagents become available. The groups of viruses which have been detected in water by rapid methods are listed in Table 2, along with the methods which were used.

A number of rapid methods have been used to detect enteroviruses in water. Immunofluorescence of infected cells, by a direct IF assay, has been used to detect poliviruses in sewage (Katzenelson and Kedmi 1979). Approximately half of the samples were negative for polioviruses by IF and positive for virus by plaque assay. This could be explained in part by the tenfold larger volume tested by plaque assay as well as by the absence of polioviruses from the samples. More recently, we have assayed sewage samples for virus by an indirect IF assay (Guttman-Bass et al. 1981) for coxsackievirus B 5, echovirus 7, and poliovirus 1 (N Guttman-Bass and Y Tchorsch, unpublished results, 1983). Virus was detected by plaque assay in unconcentrated and concentrated sewage samples at concentrations of up to 5,000 pfu/l. Fluorescent plaques of poliovirus were detected in one of the two sewage samples, but the concentrates were toxic for the cell layer. Neither of the other two viruses was detected.

These studies indicate some of the difficulties in the detection of enteroviruses by assays requiring a prior choice of antibody, limiting the range of detection. Unless the antiserum used has a broader specificity, this approach does not provide an attractive alternative to standard tissue culture methods for these viruses. Recently, antibodies which reacted with a number of enteroviruses were prepared using heat-disrupted virions as immunogens (Hasegawa and Inouye 1983). The use of such antisera, if reactive in an IF assay, might provide a less limited rapid assay for these viruses.

Table 2. Field studies using rapid methods

Virus group	Method used
Enteroviruses	IF, ELISA, RIA, EM, IEM
Rotaviruses	IF, ELISA, EM, IEM
Reoviruses	IF
Adenoviruses	EM

Hepatitis A virus (HAV), an enterovirus, is not easily isolated in cell cultures, although in combination with antibodies it can be detected. Due to the long incubation peroid of the disease, rapid detection of the virus in water followed by the introduction of public health measures might reduce the severity of a subsequent outbreak. Thus, a number of laboratories have chosen to look for this virus using rapid methods, since other methods of detection are too slow or not feasible.

HAV has been detected in sewage by a combination of affinity chromatography followed by IEM (Elkana et al. 1983). The one sample positive for HAV was from a broken sewer in a neighborhood with cases of infectious hepatitis, but the virus was not found in sewage from other nearby sites. The sample was also positive for virus by plaque assay. This method was also used to detect HAV in the Jerusalem sewage samples described above, but no HAV was found in 50 ml of sewage (N Guttman-Bass and Y Elkana unpublished results, 1984).

RIA was used to assay for HAV in samples of well water and sewage from a community undergoing an outbreak of gastroenteritis and hepatitis (Hejkal et al. 1982). HAV was found in three sewage samples and one well water sample taken a month prior to the outbreak of infectious hepatitis. Coxsackie B viruses were most frequently isolated from the water samples, and were implicated as the cause of the gastroenteritis outbreak.

ELISA has also been used to detect HAV in wastewater (Ryvarden 1982), but the relative levels of other viruses in the samples was not reported.

The importance of selectively assaying for HAV to the exclusion of a more general viral assay has yet to be demonstrated. It might be better viewed as a supplementary assessment of viral pollution and might aid in the timely immunization of the exposed population. It should be noted that the methods used for HAV detection in water did not determine viability. In all of the samples assayed by tissue culture methods as well, viable virus was found. The absence of HAV does not necessarily imply the absence of other pathogenic viruses, and HAV does not appear to be present consistently enough to warrant its use as a general virus indicator.

Rotaviruses have been the subject of increasing use of rapid methods for their detection. Since the strains cross-react antigenically, antiserum against all of the human types are relatively easy to obtain, and even cross-reacting animal strains can be used for antibody production. In addition, kits are available commercially for antigen detection. Although recently developed methods have made the direct isolation of rotaviruses from clinical specimens by tissue culture methods possible (Wyatt et al. 1983), the ease, sensitivity, and rapidity of immunoassays have made them the more common approach for isolation from water.

In particular, immunofluorescence of infected cells is a promising approach. Rotavirus has been detected in Texas sewage by idirect IF (Hejkal et al. 1982) and in drinking water samples in Mexico (Deetz et al. 1984). Using this method, Smith and Gerba (1982) detected rotavirus foci in seven secondary treated wastewater samples (0.4 ml tested), four of which were negative for viruses by plaque assay (6 ml tested). IF has also been used to quantitate the amount of rotavirus in Houston, Texas, sewage over the course of a year, with rotavirus concentrations ranging from 1 to 321 foci/l, while enteroviruses ranged from 7.5 to 800 pfu/l (Hejkal

et al. 1984). The ELISA method was also tested, but did not correlate well with the IF results and resulted in a false positive rate of 23%.

Greater numbers of rotaviruses (detected by IF) than enteroviruses have been reported for polluted estuary waters in Texas, with rotavirus concentrations of up to 4,980 foci/378 l of water (Metcalf et al. 1984).

Monoclonal antibodies have been used in IF and immunoperoxidase procedures for rotavirus detection (SA Sattar and RA Raphael, personal communication, 1984). Sewage, effluent, and river water samples were successfully assayed for rotavirus. It would be of interest to compare the sensitivity of the method using monoclonal antibodies with the use of more general antisera.

ELISA and EM have also been used for the detection of rotavirus in water. Sewage samples in the German Democratic Republic which were positive for enteroviruses were assayed for rotavirus by two methods. Out of six sewage samples, three were positive by ELISA and one by IEM (Walter et al. 1982). In contrast, Steinmann (1981) compared the two methods for sewage samples from West Germany and found 6 out of 24 samples positive for rotavirus, with agreement between ELISA and EM methods. The EM was performed without antibody, and the virus identified by size and morphology. Viability was not determined. In England, Goddard and Sellwood (1982) detected rotavirus in sewage by ELISA, but less frequently than enteroviruses. Finally, in a West German study (C Epp, personal communication, 1984), rotavirus was detected in two out of eight samples of water from a sewage-polluted river. The samples also contained other viruses as detected by tissue culture methods.

In summary, rotaviruses are present in high enough concentrations in sewage-polluted waters to be detected by IF, ELISA, and EM methods. However, only the IF method detected rotaviruses in samples negative for enteroviruses, and this method would appear to be the most sensitive for rotavirus detection.

Other viruses have also been detected by rapid methods, although they have received less attention than the above viruses. Reoviruses, which in some cases are found in high concentrations in water, have been detected in sewage by IF (Ridinger et al. 1982). The IF method compared favorably to plaque assay, both in sensitivity and in rapidity of the assay. The plaque assay isolated nonreoviruses as well, and was less diagnostic for reoviruses. The detection of reoviruses by immunological methods would appear to be a promising approach.

In a study using EM to detect viral pollution of bore water samples on Norfolk Island, Australia, viruses were identified by their morphology (Murphy et al. 1983). Out of 32 samples, 1 was positive for rotavirus, 1 for adenovirus, and 2 contained "small round virus" particles. Tissue culture isolates were also made, and poliovirus type 1 and adenovirus (types 1 and 5) were isolated. Poliovirus was found in three samples which would have been acceptable by bacteriological standards. Although the EM did not test the viability of the viruses, it was broad enough to detect virus types which might not otherwise have been found.

Prospects

The use of rapid methods for virus detection in water has made possible the detection of important viruses such as HAV and rotavirus. Such methods are still problematic for the majority of the enteroviruses and will continue to be so until the introduction of rapid assays which can detect a wider range of such viruses. One direction might be in the area of nucleic acid hybridization. Recently, a cDNA probe to coxsackievirus B 3 was used in a spot hybridization test to detect cells infected with other enteroviruses, including coxsackie A, echo, and polioviruses (Hyypia et al. 1984). Another approach is that suggested by the work of Deng and Cliver (1984), who used pooled human immune serum globulin in an ELISA to detect human enteric viruses in an additive fashion.

The development of rapid methods for adenovirus detection in water, either by immunossay or nucleic acid hybridization, is warranted to further explore their presence and distribution in the water environment.

The results of assays for rotavirus are accumulating, and this group of viruses appears to be present in high concentrations in water. Sensitive methods such as IF which are rapid and quantitative are in use to study these viruses in natural waters.

In summary, tissue culture methods, although in some cases still the method of choice for the detection of certain viruses, are being supplemented with rapid methods to detect a broader spectrum of viruses. At this point, rapid methods cannot completely replace more standard, if slower, methods of virus detection in water. However, as reagents and assays become available for the detection of more virus types by rapid methods, they will eventually be applied to the study of viruses in water.

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