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The following is an electronic text form of CHAPTER 1 of the USEPA MANUAL OF METHODS FOR VIROLOGY - EPA publication EPA/600/4-84/013. The hardcopy form of this chapter can be obtained by contacting:

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Date of Publication: February 1984

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CHAPTER 1

INTRODUCTION

1. PERSPECTIVES IN ENVIRONMENTAL VIROLOGY

The ability to multiply, to direct processes in the cells they infect, and the ability to mutate are the only characteristics of life that the virus is capable of manifesting. In essence, the virus is alive only when it infects. Outside of living cells, the virus is inert. Its essential viability in the hostile environment outside the cell is time-marked. Yet, among those viruses excreted by infected people into sewage discharged into rivers, streams, and lakes, many often survive to reach the water intakes and recreational areas of downstream communities. If that sewage or its treated effluents or sludges are discharged to the land instead, sufficient numbers of viruses may survive to contaminate crops or ground waters in the aquifers below. If the discharge is to the oceans, viruses may contaminate recreational beach waters or approved shellfish harvest waters. Over the years, cases of such contamination have been documented repeatedly even in the apparent absence of indicator bacteria.

The smallest numbers of viruses detectable in cell cultures, the most sensitive hosts for many viruses, may be sufficient to infect susceptible individuals who consume them. Thus, any number of viruses that reaches a water intake or that is consumed by a recreationalist is a potential hazard. To detect such small numbers of viruses in water requires concentrating viruses from large volumes of water.

In the past several years, a growing awareness of the waterborne virus problem has developed within the scientific community. This awareness has resulted in the development of a number of techniques for recovering viruses from waters of various qualities. These waters range from sewage to tap water. The techniques that have been developed include filter adsorption-elution, glass powder adsorption-elution, ultrafiltration, polyelectrolyte adsorption, aluminum hydroxide adsorption, protamine precipitation, hydroextraction, two-phase separation, organic flocculation, and alginate membrane filtration. Some of these methods are modestly efficient in limited circumstances. None of them has universal potential at present. There is endless change in the chemical quality of waste and receiving waters, and the unpredictable effects of such change on the efficiencies of the methods for quantitatively concentrating viruses from waters is a problem that may long be with us. Thus, methods may always require selection and flexibility to meet the needs of changing situations. Guidance for such selection and flexibility is given herein.

2. THE VIRUSES IN ENVIRONMENTAL WATERS

Enteroviruses (polioviruses, coxsackieviruses [groups A and B], echoviruses, and hepatitis A virus), rotaviruses and other reoviruses (Reoviridae), adenoviruses, and Norwalk-type agents -- a total of more than 100 different serological types -- constitute the major enteric virus complement of human origin. Most of these viruses have been detected in sewage and in receiving waters over the years. Members of other virus groups have been recovered from human feces and urine, but none has been reported with great frequency or in large numbers in sewage or in receiving waters. Viruses of non-human sources abound in environmental waters. Some of these viruses, such as reoviruses, may infect man; the significance of certain other viruses from non-human sources is as yet undetermined.

The numbers of viruses detected per liter of sewage range from less than 100 infective units to more than 100,000 infective units. In temperate climates, the numbers generally increase in the warmer months and decrease in the colder months, reflecting overall infection and excretion patterns in the community. In the tropics, the numbers of viruses in sewage are highest during the rainy season. Since viruses do not multiply outside of susceptible living cells, dilution in hostile receiving waters and the toll of time eventually reduce the numbers of viruses to levels often barely detectable by the best techniques available, even when 1,000-L quantities of water are tested. In receiving streams, however, such numbers of viruses, in terms of the daily water intake requirements of

even small communities, are not small.

When one considers the low efficiencies of the methods that we have for concentrating these viruses, that the cell culture systems used for detecting viruses are usually sensitive to less than half of the virus types excreted by man, that the plaque procedure usually used for detecting and quantifying viruses is itself relatively inefficient, and that there are undoubtedly viruses in sewage that have not yet been detected and identified, it seems reasonable to surmise that the numbers of viruses we now detect in environmental waters are probably an order of magnitude or more below the quantities actually present there. The numbers of viruses that reach recreational waters and intakes downstream of outfalls may thus be very large indeed.

3.CONCLUSIONS AND RECOMMENDATIONS OF THE WORLD HEALTH ORGANIZATION (WHO) SCIENTIFIC GROUP ON HUMAN VIRUSES IN WATER, WASTEWATER AND SOIL (from: Human Viruses in Water, Wastewater and Soil, Report of a WHO Scientific Group, Technical Report Series 639. World Health Organization, Geneva, Switzerland, 1979. 50 pp)

In 1979, the World Health Organization (WHO) published the report of a WHO Scientific Group on Human Viruses in Water, Wastewater and Soil. The Group included USEPA participation. The Conclusions and Recommendations of the Group follow and are quoted directly:

3.1 [Conclusions]

While bacterial contamination of water and soils and the associated health risks have been thoroughly studied, attention is now increasingly being focused on the hazards associated with virus contamination of water. The Scientific Group reviewed the current state of knowledge on the subject and concluded that the contamination of water and soil by wastewater and human faeces containing enteric viruses may pose real public health problems. This is also applicable to areas of the world in which the major waterborne bacterial diseases have been brought under control.

There are over 100 different types of enteric viruses, all considered pathogenic to man. Their concentration in wastewater may reach 10,000-100,000/L, and they have the ability to survive for months in water and in soil. In some instances, the ingestion of a single infectious unit can lead to infection in a certain proportion of susceptible humans.

On numerous occasions viral hepatitis A epidemics have been

waterborne. Many outbreaks of viral hepatitis A have resulted from eating shellfish grown in sewage-contaminated estuarine and coastal waters. It is also probable that a significant proportion of the reported waterborne gastroenteritis outbreaks of nonbacterial etiology have been associated with waterborne viruses (e.g., rotaviruses).

While the Scientific Group recognized that massive waterborne outbreaks of virus-associated diseases have been detected only on limited occasions, it concluded that the constant exposure of large population groups to even relatively small numbers of enteric viruses in large volumes of water can lead to an endemic state of virus dissemination in the community, which can and should be prevented.

Bacteria used as conventional indicators to evaluate the safety of potable water supplies have been shown to be significantly less resistant than viruses to environmental factors and to water and wastewater treatment processes. As a result, enteric viruses may be present in water that manifests little or no sign of bacterial pollution.

Where surveys have been carried out, viruses have been detected in the drinking-water supply system of a number of cities, despite the fact that these supplies have received conventional water treatment, including filtration and disinfection, which are considered adequate for protection against bacterial pathogens. Plans for the recycling of wastewater for domestic consumption are being considered in some cities, while many others are drawing their water supply from contaminated surface sources carrying a significant proportion of wastewater. In both situations the risk of viruses penetrating the supply system must be carefully evaluated so that adequate monitoring and treatment can be provided.

Methods for the concentration and enumeration of viruses in large volumes of water have been developed but are not yet standardized. Through the use of such methods large water samples can be monitored for viruses on a routine basis.

Water treatment methods capable of accomplishing effective virus removal and inactivation are now available, so that conventional water treatment plants can be suitably modified to deal with this problem. The formation of carcinogenic compounds when water containing organic material is chlorinated may give rise to a potential health problem. However, in situations in which there is a risk of waterborne communicable disease there should be no hesitation in continuing current water disinfection with chlorine until alternate techniques for effective virus inactivation are developed.

Viruses present in wastewater and sludge applied to land for irrigation, fertilization or disposal purposes can survive in soil for periods of weeks or even months. Edible crops, contaminated either by contact with virus-laden soil or by wastewater sprinkler-irrigation, can harbour viruses for sufficient periods of time to survive harvesting and marketing, and thus their eventual consumption constitutes a potential health risk.

Only limited data are available on the health risks resulting from the dispersion of viruses in aerosols created by sewage treatment and land disposal systems. However, a potential hazard does exist and steps to reduce it may be warranted. Disinfection of effluent prior to land disposal, particularly in the case of sprinkler-irrigation in the vicinity of inhabited areas, could be an effective preventive measure.

3.2 [Recommendations]

(1) Wherever possible, drinking-water should be free from human enteric viruses. To ensure that this goal is being achieved, a 100-L to 1,000-L sample should be tested by the most sensitive method available. In all cases of intentional direct wastewater reuse for domestic consumption, this procedure should be considered essential and should be applied at least in large urban areas in which potable supplies are derived from virus-polluted sources, such as surface water containing a significant proportion of wastewater either untreated or insufficiently treated to inactivate viruses. Further consideration should be given to the establishment of recommended virus concentration limits for water for recreational purposes, and wastewater effluent and sludge for agricultural use.

(2) Where virological facilities can be provided, it is desirable to monitor wastewater effluents, raw-water sources and drinking-water for the presence of viruses. This will provide baseline data to evaluate the health risk faced by the population.

(3) In the light of the greater resistance of many enteric viruses to disinfection and other treatment processes compared to that of bacteria utilized as pollution indicators, drinking-water derived from virus-contaminated sources should be treated by methods of proved high efficiency for removing or inactivating viruses and not only bacteria. Particular emphasis should be given in such cases to ensure the effective disinfection of drinking-water with, for example, free available chlorine residuals of 0.5 mg/L maintained for a contact time of 30-60 minutes or an ozone residual of 0.2-0.4

mg/L maintained for 4 minutes.

(4) Because of the ability of viruses to survive for long periods in seawater, it is recommended that coastal bathing and shellfish growing areas should be protected from contamination by wastewater and sludge. Virus monitoring of these areas is a desirable measure.

(5) Control procedures should be instituted in all situations in which wastewater or sludge is used for irrigation or fertilization, to prevent the contamination of vegetables and fruits which are to be eaten raw. (Moreover--even though they may eventually be cooked--contaminated raw vegetables are liable to pollute other food in the kitchen.) Where it is nevertheless planned to irrigate such crops or where sprinkler-irrigation is to be used near populated areas, the effluent should be treated so that it reaches a high microbiological quality approaching that of drinking-water.

(6) Since the factors that influence the movement of viruses in soil are still not fully understood, and since effluent and soil conditions vary so greatly, caution should be exercised if wastewater irrigation or land disposal takes place in the vicinity of wells supplying drinking-water. Careful study of local conditions is required and the cautious siting of such wells and routine virological monitoring of the water are advised as safety measures.

(7) Further research is necessary into the health risks associated with viruses in water and soil. These studies should include the development and evaluation of methods of detecting viruses and alternative indicators of virus pollution (e.g., phages) and the improvement of treatment methods for the inactivation and removal of viruses from water and wastewater. The dissemination and survival of viruses in the natural environment should also be investigated.

(8) A standard method should be developed for the concentration and detection of viruses in large volumes of drinking-water (e.g., 100-1,000 L) based on a full evaluation in different laboratories of present techniques. Such an attempt would facilitate the development of virus-monitoring programmes and would ensure a maximum degree of comparability of results. A laboratory quality-control system should be developed to enable participating laboratories to standardize their procedures.

3.3 Summary

Although not a direct response to the efforts of the WHO Scientific Group, this manual should make possible the

monitoring operations envisioned by that group.

4. HISTORY OF METHODS SELECTION

In 1965, a symposium on Transmission of Viruses by the Water Route included a major segment on methods for recovering viruses from the water environment. The focus on methods, within the context of the water transmission problem, resulted in a growing interest in methods research over the years that followed.

In 1975, a WHO Working Group on Bacteriological and Virological Examination of Water (see: Report of a Working Group on Bacteriological and Virological Examination of Water - World Health Organization in collaboration with the Federal Republic of Germany, Mainz, Germany, April 21-25, 1975. Water Research, 10:177-178, 1976. also see: Lund, E. 1982. Virological Examination, 3:462-509. In Suess, M. J., ed., Examination of Water for Pollution Control, Pergamon Press, New York.) met in Germany to recommend the promulgation of methods for recovering bacteria and viruses from various environmental waters and sludges. The USEPA participated. Although methods for recovering bacteria are well-advanced, methods for recovering viruses are not. Nonetheless, the Sub-group on Virological Examination, with some reservations, selected several methods for promulgation which it believed were the best methods currently available. (The mandate of the sub-group did not include tap and ocean waters, but some of the methods described herein are directly applicable to such waters.) The American Public Health Association, The American Water Works Association and the Water Pollution Control Federation, through their jointly published STANDARD METHODS, and the American Society for Testing Materials have also recommended methods for recovering viruses from the water environment. The methods described in this USEPA manual have seen the benefit of the research and experience of the years that have passed since 1965. Nonetheless, the current state-of-the-art requires that the following caveats are considered:

Changes in the quality of waters sampled may affect markedly the efficiency of each method described. Few studies are available that compare the efficiency of one method with another under the same conditions.

None of the methods described has been studied with more than a few virus types. Most studies have been laboratory and not field studies. None of the methods is equally efficient for the recovery of all of the types of viruses frequently found in environmental waters.

Some of the techniques described are labor-intensive. Some require expensive equipment. In a methodology so rapidly evolving, there is a risk of obsolescence and obvious economic consequences.

4.1 Recommendations of the WHO Working Group and the WHO Scientific Group

Both the aforementioned WHO Working Group on Bacteriological and Virological Examination of Water and the WHO Scientific Group on Human Viruses in Water, Wastewater and Soil suggested tentatively for concentrating viruses from 0.2- to 5-L volumes of wastewater and other waters a microporous filter adsorption-elution technique, adsorption-precipitation with various salts, polyethylene glycol hydroextraction, aqueous polymer two-phase separation, and soluble alginate filtration. These Groups tentatively suggested tangential flow ultra-filtration and flow-through adsorption-elution systems for concentrating viruses from 5- to 400-L volumes of relatively clean waters.

The WHO Groups also recommended tentative methods for recovering viruses from solids in waters and from sludges. These methods were based on elution, with beef extract, serum, or other proteinaceous materials, of viruses from the solids. The tentative methods recommended by the two WHO Groups have not been presented yet as operational procedures that can be followed readily in the laboratory. Several of those methods (but not the subsequent viral assays) are intended for use in bacteriological laboratories that are minimally equipped and staffed. Both Groups recommended that the tentative methods undergo round-robin (done under identical conditions by several participating laboratories to determine the effectiveness, precision, and accuracy of a method) testing.

4.2 Recommendations in STANDARD METHODS (Standard Methods for the Examination of Water and Wastewater, 15th Edition. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C., 1981) for Detecting Viruses in Various Waters

The 15th edition of STANDARD METHODS presents a microporous filter adsorption-elution technique, an aluminum hydroxide adsorption-precipitation technique, and a polyethylene glycol hydroextraction technique, all as tentative standard methods for recovering viruses from waters and wastewaters. The filter adsorption-elution technique is recommended for concentrating viruses from only a few liters of any water (single-stage filter adsorption-elution technique) and from large volumes of purer waters (two-stage filter adsorption-elution technique).

The latter technique may be used to concentrate viruses from volumes of 1,000 L and more of finished waters. STANDARD METHODS recommends the aluminum hydroxide adsorption-precipitation technique and the polyethylene glycol hydroextraction technique only for small volumes of waste and other relatively highly contaminated waters.

The STANDARD METHODS procedures have not been round-robin tested.

The 15th edition of STANDARD METHODS does not recommend methods for recovering viruses from solids in water or from sludges, but it does describe virus assay procedures.

Although the methods in STANDARD METHODS have been written in a manner intended as procedural, STANDARD METHODS recommends that testing with these methods should be done only by competent and specially trained water virologists having adequate facilities.

4.3 Recommendations of the American Society for Testing Materials (ASTM)

Most of the methods described in this USEPA manual have been round-robin tested by the ASTM. A formal acceptance of these methods as ASTM methods is pending.

5. THE USEPA MANUAL

The USEPA manual contained herein is state-of-the-art. The manual comprises the best methodology available today, and it will be revised frequently so that it remains state-of-the-art.

Each method in this manual has been presented as a step-by-step procedure that should be easily followed by technicians trained in bacteriology and familiar with aseptic techniques and safety procedures. Each method has been subjected to numerous successful laboratory simulations by both experienced and inexperienced technical personnel. Only the assays for viruses, which must be done in cell cultures or in animals, require the skills of trained virologists.

This manual makes it possible for any competent water bacteriology laboratory that can arrange for viral assays (and identifications) by a competent virology laboratory to concentrate and recover viruses from waters and from sludges and other solids.

6. BIBLIOGRAPHY

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