Methods in Environmental Virology

Methods in ENVIRONMENTAL VIROLOGY

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Preface

A great deal of progress has been made in the last decade in the development of methodology for detecting viruses in the environment. The use of these methods during the next decade will result in a greater understanding of the epidemiology of environmentally transmitted viral disease. Progress has been rapid in recent years on the development of methods for viral concentration, isolation, and detection from the environment. Every month is witness to reports on new methods or improvement of old ones.

The purpose of this book is to put into one document a concise review of available methodology for studying viruses in the environment. Because methodology will evolve as new techniques become available, emphasis has been placed on strategies for isolating viruses from a particular environment. Each environment presents its own difficulties, and so the approach to concentration and/or isolation must often be different.

With the basic tools now provided for studying viruses in the environment, increased efforts are now possible for monitoring viruses in the environment. It is hoped that this book will be a starting point for those who now must be concerned with such work.

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Contents

Preface
Contributors

1 INTRODUCTION TO ENVIRONMENTAL VIROLOGY 1

V. Chalapati Rao

I Importance of Studying Viruses in the Environment 1
II Virus Transmission by Water and Wastewater 2
III Virus Removal from Water and Wastewater 5
IV Virus Transmission Via Foods 7
V Virus Transmission by Aerosols 7
References 9

2 LABORATORY METHODS FOR THE GROWTH AND DETECTION OF ANIMAL VIRUSES 15

Eric M. Smith and Charles P. Gerba

I Introduction 15
II Cultivation of Virus Isolates 15
III Detection and Identification of Viruses 20
IV Selected Technical Aspects Involved with Virus Detection Procedures 34
References 39

3 PRESERVATION OF ENVIRONMENTAL SAMPLES 49

Sagar M. Goyal

I Introduction 49
II Factors Affecting Virus Survival During Storage 50
III Preservation of Environmental Samples for Virological Analysis 52
References 56

4 CONCENTRATION OF VIRUSES FROM WATER BY MEMBRANE FILTERS 59

Sagar M. Goyal and Charles P. Gerba

I Introduction 59
II History 60
III Virus Concentration from Different Waters 72
IV Factors Influencing Virus Concentration by Microporous Filters 79
V Reconcentration 88
VI Methodology for Concentration of Enteroviruses from Water 91
References 109

5 METHODS (OTHER THAN MICROPOROUS FILTERS) FOR CONCENTRATION OF VIRUSES FROM WATER 117

Samuel R. Farrah and Gabriel Bitton

I Introduction 117
II Methods Used Primarily for Concentrating Viruses Directly from Water Samples 118
III Methods Used for Concentration of Viruses from Relatively Small Volumes of Water or as a Second-Stage Concentration Procedure 129
IV Methods Used in Limited Studies on Concentration of Viruses from Water 138
V Summary 140
References 144

6 DETECTION OF VIRUSES IN SOIL AND AQUATIC SEDIMENTS 151

Charles P. Gerba

I Isolation of Viruses from Soils 151
II Isolation of Viruses from Aquatic Sediments 152
References 158

7 ISOLATION OF VIRUSES ASSOCIATED WITH SLUDGE PARTICLES 161

Samual R. Farrah

References 169

8 DETECTION METHODS FOR VIRUSES IN SOLID WASTE LANDFILL LEACHATES 171

Mark D. Sobsey

I Introduction and Background 171
II Equipment and Apparatus 174
III Procedures 175
References 177

9 DETECTION OF VIRUSES ON FOMITES 179

Beatrice L. England

I Introduction 179
II History: Epidemiological Evidence of Viral Disease Transmission by Fomites 181
III Documentation of Viral Recovery from Fomites 184
IV Control of Fomite Transmission of Viruses 195
V Factors that Influence the Survival of Viruses on Fomites 200
VI Methodology for Recovery of Viruses from Fomites 209
References 215

10 DETECTION OF VIRUSES IN FOODS 221

Edward P. Larkin

I Introduction 221
II Limitations of Virus Detection Systems 222
III Public Health Significance of Viral Isolates 223
IV Food Complexity 224
V Factors to be Considered in Method Development 225
VI Selection of Food for Analysis 225
VII Methodology 229
VIII Enumeration and Identification of Isolates 232
IX Summary 234
References 235

11 DETECTION OF VIRUSES IN SHELLFISH 243

Mark D. Sobsey

I Introduction and Background 243
II Enteric Virus Concentration from Shellfish 244
III Procedures for Isolating and Quantifying Enteric Viruses in Shellfish Concentrates 252
References 256

12 METHODS FOR CHARACTERIZATION OF VIRUS AEROSOLS 261

J. Clifton Spendlove and Kerby F. Fannin

I Introduction 261
II Virus Aerosols-Occurrence and Significance 262
III Influence of Environment on Survival of Viruses in Aerosols 265
IV Methods for the Study of Virus Aerosols 273
V An Approach to the Study of Environmental Virus Aerosols 303
VI Virus Aerosol Models 311
References 316

13 EVALUATING CHEMICAL DISINFECTANTS FOR VIRUCIDAL ACTIVITY 331

Charles Howard Stagg

I Introduction 331
II Viral Inactivation 332
III Influencing Factors 338
IV Experimental Methodology 343
References 345

Author Index 349
Subject Index 373

1

Introduction to Environmental Virology

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I. IMPORTANCE OF STUDYING VIRUSES IN THE ENVIRONMENT

Four decades ago, when the first sewage effluent sample was collected by Melnick in the East River of New York City [1], his concern was to delineate some correlation between the occurrence of viruses in sewage and the presence of cases in the community. Results of his study sowed the seed for environmental virology, which has attained the status of a recognized discipline.

Studies on transmission of viruses by the water route received considerable thrust in different parts of the world after the 1955–1956 epidemic of waterborne infectious hepatitis in New Delhi, India, in which 30,000 cases, including 73 deaths, were reported [2]. The deliberations of the 1965 symposium on "Transmission of Viruses by the Water Route" in Cincinnati, Ohio [3], highlighted the significance of the problem and urged concerted efforts to develop adequate quantitative methods for detecting low-level virus transmission through water, to verify the concern whether viruses can survive undetected under conditions which eliminate coliform organisms, to develop sound judgments of what constitutes an infective dose of virus to humans, to study virus persistence in the deliberate reuse of wastewater, and to intensify efforts to detect the agent of infectious hepatitis. Nearly a decade later, in 1974, scientists at an international conference on "Viruses in Water" in Mexico City [4] proposed that it was time to recommend consideration of virus standards for drinking and other waters.

The World Health Organization recognized the growing importance of the problem, organized an in-depth discussion on human viruses in water, wastewater, and soils and published a report in 1979 [5] to create interest among those responsible for public health and economic planning both in the developing and developed countries. This report made "an assessment of the public health importance of viruses in water, wastewater, and soils and of the nature of risks for exposed persons; it relates to the methods available for monitoring viruses in different situations and identifies areas for further research."

As a result of the above sustained efforts, today there is an increasing awareness that human populations around the world are exposed to waterborne viruses through the consumption of contaminated water, shellfish, and crops; as a result of recreational activities involving water; and by exposure to aerosols during land application of wastewater.

Environmental virology deals with viruses of human origin in three important segments of the environment: water and wastewater, food, and air. Considerable information has been generated through nearly four decades on the occurrence and survival of viruses in water and wastewater; only limited information is available on viruses in foods. This disproportion may be due to the limited number of investigators in the food field, lack of tested and proven methods, and lack of epidemiological data concerning their public health significance. However, it is speculated that viruses may be contributing to disease in man via food, and this possibility can be substantiated only as more information becomes available.

Human enteric viruses in aerosols have become a matter of concern only recently. But only when quantitative methods to detect viruses in aerosols are sufficiently developed and tested in different geographic areas can we develop an adequate understanding of their dissemination in air and their potential hazard to human health.

II. VIRUS TRANSMISSION BY WATER AND WASTEWATER

The significance of human enteric viruses in water was brought out effectively by Plotkin and Katz at the 1965 symposium [6] when they surmized, based on a review of the literature, "that one infective dose of tissue culture is sufficient to infect man." Based on this assertion, Berg [7] concluded that "any amount of virus in drinking or recreational water that is detectable in appropriate cell cultures constitutes a hazard to those drinking such water."

Over 100 different types of human enteric viruses have been reported to occur in the feces of humans and thus could eventually gain entrance into sewage and polluted surface water. The concentration of enteric viruses in human feces may be as high as 10^4-10^6 PFU per gram [8]. Rotaviruses up to 10^9 particles per gram of feces were demonstrated [9]. Their numbers in raw sewage vary greatly, depending on several factors such as level of hygiene of the population, incidence of disease in the community, socioeconomic lével, and season of the year. Accordingly, enteric virus densities reported in raw domestic sewage are 100–200 PFU per liter in Houston [10,11], 1,000–11,500 PFU per liter in Nagpur, India [12], 180–463,500 PFU per liter in South Africa [13], and 6000–1,060,000 PFU per liter in Haifa, Israel [14].

Peak levels of enteroviruses in sewage occur in the late summer and early fall in the temperate climates, whereas in tropical countries like India the highest numbers were encountered in the rainy season and the lowest numbers in winter [15]. Enteric viruses survive the conventional secondary treatment of sewage, including chlorination, and as a consequence viruses have been detected in rivers and streams.

There are limited data on the occurrence of viruses in drinking water. Of 200 samples, 18% were positive for virus in a study in Paris [16]. Enteric viruses were isolated on several occasions from the water distribution system in Russia [17,18], with the water meeting national bacteriological standards. Viruses have also been isolated by Nupen [19] from treated drinking water samples which did not contain fecal coliforms in Africa. In Nagpur, India, 7 of 50 samples of treated drinking water in the distribution system which were negative for coliforms and which contained 0.2–0.8 mg/liter of total chlorine yielded 1–7 PFU per 30–60 liters. In a Rumanian study [20] coxsackieviruses were detected in 2 of 65 drinking water samples, and poliovirus has been detected in drinking water containing total chlorine levels of 1.3–1.7 mg/liter in the United States [21]. Twelve of 18 samples in Israel yielded polioviruses and echoviruses [22], and from Mexico, coxsackie B virus was detected in 8 of 11 [23] and rotavirus in 11 of 11 tapwater samples [23].

Studies cited above indicate that fecal bacteria, which traditionally were relied on to indicate pollution (and still are used in evaluating the safety of most potable water supplies), are now recognized as inadequate indicators of the presence of viruses in waters in many cases.

Wherever viruses are present in high concentrations in drinking water, outbreaks have occurred involving large numbers of people. Such situations, like the New Delhi epidemic of infectious hepatitus in 1955–1956 [2], are rare. What is more significant is the low-level virus spread that takes place even through treated drinking water. The significance of low concentrations of viruses in treated drinking water is analyzed [5] and the following hypothetical example given:

"In a city of 1,000,000 population consuming water treated conventionally but insufficiently to remove all viruses, the expected concentration of virus might be 1 infectious unit per 20 liters of drinking water. This situation could give rise to the following circumstances: assuming each person drinks about 1 liter of water daily, then each day an average of 50,000 persons would ingest at least one infectious virus particle in their water. Conservatively, because of immunity and other host resistance factors, one can assume that only 1% of those exposed would be infected—i.e., 500 persons per day or 182,000 (500 X 365) persons per year. Assuming that only 1 in 50 persons infected would become ill, 3650 persons would have obvious clinical disease per year characterized by a broad range of symptoms caused by the enteric viruses. In addition to this burden of illness, the 182,500 persons could act as carriers who in turn might infect their contacts."

This kind of dispersal of virus could be responsible for a proportion of the endemic viral diseases in communities drinking treated waters. What would be the magnitude of exposure to enteric viruses in a large proportion of people in rural areas of developing countries and consuming untreated water from tanks, canals, streams, and shallow wells? The defecating habits of people in villages and small towns in open fields and on banks of canals and lakes aggravate the situation in that during the rainy season, fecal deposits are continually washed into subsurface water, improperly constructed wells, and lakes.

Among the various viral diseases transmitted by drinking water, outbreaks of infectious hepatitis stand prominent owing to the explosive nature of the outbreaks and characteristic symptomatology. Other waterborne virus disease outbreaks are not as easily recognized because clinical symptoms appear in a small proportion of infections, and far more frequently the infections are subclinical and hence difficult to trace or document. Further, the spectrum of disease syndromes caused by enteric viruses is so wide that scattered cases of illness cannot be attributed to a single etiological agent.

Swimming-pool-associated pharyngoconjunctival fever, caused by an adenovirus, is now well recognized; pools containing no free residual chlorine allow the survival and accumulation of these viruses. Other waterborne enteric viruses are also suspected as a hazard to bathers, though the risk is lower than would arise from drinking such water. For example, enteroviruses such as coxsackie B1 from recreational pools in Toronto [24] and echovirus types 3 and 11 from wading pools in Albany, New York [25], have been isolated.

Another area of concern is the discharge of human viruses into the marine environment through sewage outfalls and polluted rivers. The incidence of suspected viral gastrointestinal diseases is higher in swimmers in polluted seawater when compared to nonswimmers or to those who swim in unpolluted seawater [26]. Enteric viruses have been found at bathing areas in coastal waters which met a bacteriological standard of less than 1000 coliforms per 100 ml [27]. Polluted coastal water has always been a danger to the shellfish growing areas. Shellfish harvested from these areas and consumed raw or inadequately cooked have caused numerous outbreaks of viral hepatitis [28]. This observation infers that this virus persists for a long time in sewage, seawater, and seafood and thereby gets transmitted to humans through the marine environment.

The association of viruses with suspended solids in sewage effluents and natural waters has created additional problems. Viruses persist in sediments and are likely to reappear in the overlying water through changes in the physicochemical conditions of the water, such as changes in water velocity due to rainfall or impoundment discharge, heavy precipitation causing a reduction of the cationic strength of water, and competitive interactions of organic materials introduced from land runoff and domestic and industrial waste effluents [29]. It has been demonstrated that solids-associated virus appears to survive longer and requires higher doses of chlorine and longer contact times for inactivation by chlorine.

There is increasing worldwide interest in the reuse of wastewater for irrigation and for direct application to land as a measure to reduce pollution loads on heavily contaminated rivers and lakes. Enteric viruses from wastewater are recovered by adsorption to soil particles and remain viable for several months, perhaps to be released again when proper conditions for their desorption from soil particles develop. Infiltration of viruses into ground water was demonstrated at a spray irrigation site receiving secondary sewage effluents [30].

The possibility of disease transmission by aerosols from spray irrigation sites and waste treatment plants is attracting increased attention. The degree of hazard depends on several factors, including degree of wastewater treatment, extent of aerosol travel, proximity to populated areas, and prevailing climatic conditions.

III. VIRUS REMOVAL FROM WATER AND WASTEWATER

Enteroviruses do not reproduce outside a living host, and thus the quantity of virus in the environment gradually decreases over a period of time. But a quick and reliable way of reducing their numbers is by treatment of water and wastewater. Various conventional sewage and water treatment methods now in practice can remove viruses to various degrees. Virus removal during treatment occurs by two mechanisms. One is a physical separation of particles from the aqueous phase, and the second is inactivation or destruction of viral particles. Physical separation processes are sedimentation, coagulation, precipitation, filtration, and adsorption. The processes that cause inactivation are disinfection with chemical oxidants, high pH, and photooxidation by certain dyes in the presence of light.

Wastewater treatment by primary settling removes up to 50% of viruses from raw sewage owing to their association with solids [31]. Among secondary treatment procedures, the activated sludge process is the most effective biological method, removing up to 99% of the viruses present, although detectable virus still remain in the effluent. For example, at one plant in Bombay, India, the concentration of enteroviruses was 50 PFU per liter of unchlorinated effluent [32], and in two plants in Houston the concentration was 2 PFU per liter [33]. The performance of trickling filters and stabilization ponds varies, though well-designed multicellular ponds [15] can remove 80–90% of the viruses originally present.

Chemical coagulation is regarded as one of the most effective single-step treatments. Alum (aluminum sulphate), lime (calcium hydroxide), and iron salts, as well as polyelectrolytes, have been shown to be capable of removing 90–99% of the viruses suspended in water [34,35]. Lime treatment is considered most effective since it not only removes the viruses physically but also inactivates them by exposing them to a high pH. Filtration of coagulated effluents is an important additional process, slow sand filtration being more effective than rapid sand filtration. Viruses can also be removed by adsorption to a variety of surfaces like activated carbon, diatamaceous earth, coal, glass, colloidal organic matter, clays, and soils. Adsorption to such surfaces is easily reversed by altering ionic concentration and pH or by introducing competing organic matter.

The most effective way of destroying viruses in wastewater and water is to use chemical disinfectants such as chlorine, which is an excellent virucide. A number of factors such as temperature, pH, presence of organic matter, physical state of the virus, and type of virus are known to influence the effectiveness of chlorine in inactivating viruses. Sewage effluents contain large concentrations of organic matter and ammonia, and thus virus reductions are not great since chlorine combines readily with ammonia. Doses of 40 mg/liter for 10 min are necessary to bring about 99.9% destruction of the virus in sewage [36].

Such a practice involving high chlorine doses in sewage effluents creates problems of toxicity for fish and other forms of life in receiving waters. Formation of carcinogenic chlorinated hydrocarbons is viewed with great concern. Added problems in chlorination of waters are the observed variability in the resistance of different enteric viruses [37] (time required for 99.9% inactivation ranged from 2.7 min for reo 1 to 120 min for coxsackie virus A6), and the enhanced resistance of solids associated and aggregated viruses. Additional concern is expressed that polioviruses subject to sublethal exposure to chlorine develop chlorine resistance [38].

IV. VIRUS TRANSMISSION VIA FOODS

Besides water, food constitutes an important (but the least explored) vehicle in the transmission of human enteric and other viruses. Association of food with transmission of overt viral diseases is not common, but food transport of viruses to humans and animals can be very frequent. Viruses in foods can be derived from sources like sewage-contaminated water, infected food handlers, and viruses intrinsically present in animals.

A variety of viruses that infect humans have been demonstrated in food animals such as calves, cows, goats, and swine [39,40]. The same viruses have sometimes been isolated from human contacts living in close association with the animals [41]. The presence of the above human viruses in animals indicates that animal viruses may be agents of subclinical infections or disease in man.

There are a number of reports indicating the transmission of viruses by foods. Milk has been epidemiologically implicated in at least five outbreaks of poliomyelitis [42–46], and enteroviruses have been detected in raw milk and shellfish [47–49]. Hepatitis A has been shown to be transmitted by consumption of raw shellfish and foods contaminated by infected food handlers [50–52]. Three of 12 market-purchased raw ground beef samples were reported to contain polioviruses and echoviruses [53]. Vegetables grown in fields irrigated with sewage were found contaminated with polio and echoviruses [54]. Echoviruses and coxsackieviruses have been shown to persist on vegetables after 2 months of storage [55].

Polio 1 and coxsackie B1 and B6 viruses added to different commercial frozen foods and stored at room temperature, -10°C, and -29°C were still available after 1 week, 1 month, and 5 months, respectively [56]. Foot-and-mouth disease virus survived in meat for 73 days [57]. When ground beef was seeded with coxsackie A9 virus at a concentration of 9.3×10^4 PFU per gram and stored either at 4°C or 23°C, on the eighth day of storage the virus content was 7.2×10^4 at 4°C and 3×10^4 at 23°C, and 2 weeks were necessary before significant (90.8%) virus reduction was noted [58].

Viruses in large concentrations are not likely to occur in contaminated foods. As such, contaminated foods can seed infections in a small number of people, after which such infections may be spread by other routes in epidemic fashion.

V. VIRUS TRANSMISSION BY AEROSOLS

Our interest in detection, survival, damage, and inactivation of human enteric viruses in aerosols arose from the dispersion of aerosols and viruses by sewage treatment and land disposal systems and the associated health risk.

During sprinkler irrigation, which is commonly used for wastewater applications to the land, between 0.1 and 1% of the liquid is aerosolized, depending on the type of spray device, the pressure, and wind speed. Pathogenic bacteria and viruses can become entrapped in airborne water droplets ranging in size from 1 to 50 μm. Aerosols containing enteric microorganisms formed by wastewater treatment processes have been detected 1200 m downwind [59], while it has been estimated that microorganisms from sprinkler irrigation of food processing wastes might be spread as far as 25 km [60]. Teltch and Katzenelson [61] have detected enteroviruses 40–100 m downwind of sewage spray irrigation fields. They found that the detectable concentration of viable airborne microorganisms increases with an increase of relative humidity and a decrease of solar radiation. Darkness facilitates survival of aerosolized enteric microorganisms up to 10 hr more than during daytime. Of viable particles detected in their study, 30% were in the respirable size range of under 5 μm.

Humans may be infected by aerosols containing pathogenic bacteria or viruses primarily by inhalation of particles of 0.2–2 μm size which penetrate the alveoli, but larger droplets in 2–5 μm range or greater, which are trapped in the upper respiratory tract, are removed by ciliary action and may find their way into the digestive tract.

Blanchard and Syzdek [62] have shown that in droplet formation of the surface of aerated liquids, the droplet formed scavenges organic material and microorganisms; the result is that the aerosolized droplet may contain a bacterial concentration 100 times or greater than that of ambient water. This suggests that bubbles formed during the aeration of sewage treatment such as activated sludge may lead to the aerosolization of liquid droplets containing very much higher concentrations of pathogens than the wastewater itself.

Baylor et al. [63] have shown that viruses in seawater become concentrated by rising air bubbles which, on bursting at the surface, form jet droplets which can be carried to adjacent beaches and become a potential public health risk. They concluded that seawater in which raw sewage is present may produce an airborne health hazard at adjacent residential or recreational areas, even when there is no direct exposure to the pathogens by bathing.

An epidemiological study of possible health risks associated with sprinkler irrigation with wastewater was carried out in Israel [64]. In 77 agricultural settlements practicing sprinkler irrigation with oxidation pond effluent after 3–7 days of retention time, the incidence of typhoid fever, salmonellosis, shigellosis, and infectious hepatitis was from 2–4 times higher than in 130 control settlements not practicing sewage irrigation.