ENTERIC VIRUS

Collectively known as enteric viruses, there are more than 100 viral entities associated with human feces. The health significance of these agents in humans range from poliomyelitis (polio), hepatitis and gastroenteritis to innocuous infections.

Enteric viruses are extremely small particles ranging from 20 nanometers (mu) to 85 mu in diameter. In comparison, a human red blood cell averages 7,600 mu in diameter. Each virus contains a single type of nucleic acid, either RNA or DNA, which is enclosed by a protein "shell" called a capsid.

Replication of viruses can *only* take place in a living host cell. The virus is capable of using its genetic information to commandeer the host cell's machinery to produce more virus particles. In the case of enteric viruses, these particles are released into the host's feces and subsequently find their way into the external environment.

Why are enteric viruses a public health concern?

•Certain viruses can cause serious disease when ingested by susceptible individuals.

•Enteric viruses can be transmitted by the water route.

•There is lingering uncertainty as to acceptable virus levels in drinking water and concern over viruses in wastewater and reclaimed water.

•When compared to coliform bacteria, many viruses are more resistant to commonly employed water and wastewater disinfection.

Coliform bacteria are the standard for evaluating the sanitary quality of water and efficacy of treatment processes. Although many viruses may be more resistant than coliform to disinfection, coliforms remain a good indicator of undesirable bacterial contamination, and are fairly reliable as virus indicators in drinking water treatment practices. However, a great deal of effort by the scientific and professional community has been expended, and continues to be expended, to evaluate the concern over the coliform standard as an indicator of virus inactivation.

Transmission

The most common mode of Enteric Virus transmission is by person-to-person contact. Small children are the most susceptible because of their close contact with other children and their less than optimal hygienic habits. Adults are generally less subject to infection because of immunity acquired by previous exposure to the virus. Enteric viruses can be transmitted by the water route.

Diagnosis

Clinical diagnosis of a viral infection requires identification of a virus in feces or body fluids, or viral antibodies present in serum of the patient. Antibodies detected in the serum indicate a present or past infection. Active infections are determined somewhat after-the-fact by comparing concentration of antibodies from samples taken a few weeks apart (i.e. acute and convalescent phase serum samples).

Environmental Detection

In treated wastewater, a secondary effluent (activated sludge) will usually have culturable virus concentrations of less than 10 per 1,000 mL. The number of viruses may in actuality be greater, but based upon the virus assay methods presently available, these are the orders of magnitude observed.

Due to the low numbers of viruses expected in water and wastewater and restrictions in the viral assay procedure, virus concentration must be attempted. Large sample volumes, as much as 1,500 Liters, are passed through filters in such a manner that viruses present are adsorbed to the filter medium. Most often these are 10 inch spun-glass filters that are electropositive in charge and through which the sample water is passed at a rate of approximately one gallon per minute. The adsorbed viruses are then eluted from the concentrating filter using as small

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a volume as is possible and this concentrate is assayed for the presence of culturable viruses.

The virus assay is performed in the laboratory by inoculating the sample concentrate onto monolayers of tissue culture cells, the most common of which is a Buffalo Green Monkey Kidney (GBMK) cell line. If viruses are present, they will grow and destroy the host cells usually within 10 to 14 days.

One of the most common methods for the enumeration of viruses is the plaque assay. In this method, the inoculated cells are overlain with agar in order to hold the viruses in place. After suitable incubation, any culturable viruses present in the sample concentrate will begin to destroy the host cells. The destroyed cells appear as a hole or plaque in the tissue culture cell sheet. Each plaque forming unit (PFU) is the result of the presence of a single virus or clump of viruses. The plaques are counted and their number are equal to the PFU per inoculum volume. Using the appropriate adjustments the number of PFU per volume of the water sampled can be calculated.

A most probable number (MPN) method may also be employed in which destruction of the tissue culture cell sheet by cytopathic effect (CPE) takes place without the agar overlay. Dilutions of the sample are inoculated into separate flasks and general destruction of the cell sheet is observed by microscopy. The most likely concentration of viruses in the original sample is calculated based upon dilutions in flasks that show CPE.

Although the actual recovery of viruses from environmental samples cannot be measured,
laboratory studies have shown that the efficiency of virus recovery using these standard methods is 10 percent or better.

Bacteriophage

Viruses that infect bacterial cells are called bacteriophage. There are a variety of these viruses, called coliphage, which infect many subspecies of *Escherichia coli*. These phages are commonly present in wastewater in relatively large numbers as compared to enteric animal viruses. Their source is the feces of humans and animals.

A variety of coliphage are called “male specific” because they infect the bacteria via the pili (small appendages on the bacterium’s surface) and bacteria with these appendages are called “male”. These male specific phage have the same shape and size of small enteroviruses and contain single
stranded RNA. It has been noted that they demonstrate the same resistance to environmental factors, including disinfection, as do the most resistant animal enteroviruses.

In the laboratory, these viruses are detected by the formation of plaques on “lawns” of susceptible *E. coli* . This procedure is easier and less costly method than the use of tissue culture as employed with animal viruses. Results are usually available within 5 days. Because of these attributes: the relative abundance of these coliphages in wastewater; their ease in detection; and , their apparent equivalency to the survival characteristics of important enteric viruses, there is a growing interest in the use of these coliphages as indicators of the presence enteroviruses in water and wastewater. They appear to be particularly useful in evaluating the ability of water and wastewater treatment processes to remove viruses.

Further Information

For more information regarding Virus sampling, detection and current regulation, please call BioVir Laboratories at 1-800-GIARDIA (442-7342).

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