United States
Environmental Protection
Agency

Health Effects
Research Laboratory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-84-022 Jan. 1985

EPA

Project Summary

Reoviruses in Water Pollution Testing

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Removal of the outer reovirus coat using proteolytic enzymes greatly increases the infectivity of most reoviruses prepared in cell culture. The purpose of this study was to determine the potential importance of enzyme-enhanceable reoviruses in water quality control testing. Procedures were developed that would recover and quantify infectious and enzyme-enhanceable reoviruses in sewage.

Twelve cell lines were tested to determine their sensitivity to reoviruses of three serotypes that had been isolated from sewage. Madin-Darby bovine kidney (MDBK) cells were the most susceptible. Sewage-isolated, protamine-precipitated reoviruses were also used in conjunction with MDBK cells in a comparative evaluation of immunofluorescent cell count (ICC) and plaque assay procedures. Immunofluorescence assay is more sensitive and more rapid than plaque assay procedures for quantifying reoviruses recovered from sewage.

A procedure for protamine sulfate precipitation of reoviruses and rotaviruses was developed. The use of egg albumin with protamine sulfate efficiently precipitates virus present in sewage and allows assay of reovirus by immunofluorescence. The virus-containing precipitate can be collected by low-speed centrifugation rather than filtration, which allows larger sample volumes to be examined. The optimal concentration of enzyme for enhancing recovery of reoviruses and rotaviruses precipitated from sewage was 200 μg of trypsin/ml.

During a one-year period, the concentration of reoviruses was compared with the concentration of enteroviruses in sewage. The reoviruses were consistently recovered in approximately 5-fold higher concentrations than the enteroviruses. Reovirus infectivity is activated by enzyme treatment in some sewage, but not in other sewage. Some reoviruses recovered from sewage were inactivated by enzyme treatment. Additional work is needed to fully explore the conditions for enzyme enhancement of reoviruses recovered from sewage. However, even without enhancement, reoviruses were isolated from raw sewage in sufficiently high numbers to suggest that reovirus recovery procedures should be included in the virological evaluation of sewage-polluted water.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Enteric viruses are known to occur in water that receives sewage effluents and other wastewaters. There is, however, no assurance that the procedures currently being used for detecting viral pollution of water are adequate or are the most sensitive procedures available for quality control testing. If the public is to be protected from the hazards of viral disease posed by waterborne exposure, it is imperative that the best possible standard virus testing procedures be developed.

This report describes a study to evaluate reoviruses for their potential usefulness in water quality testing. The reasons for studying reoviruses were as follows: (a) They occur abundantly and consistently in sewage. (b) They are rarely known to cause disease, so are relatively safe to handle in the laboratory, and are unlikely to become targets of eradication by vaccination. Therefore, standardized tests based on reovirus detection will not have to be changed in the future. (c) They have a broad host range, so contamination by man and lower animals is detected. (d) They are amenable to low-cost isolation and identification procedures. (e) A single antiserum will detect reoviruses of all three serotypes. (f) Reoviruses are easily cultivated to high infectious titers. (g) Reovirus hemagglutination inhibition tests can be used to assay antibodies in epidemiologic studies. (h) The vast majority of reoviruses are present in a potentially infectious form which is not normally detected by conventional infectivity assays. These assays detect only the infectious form unless the virus is exposed to proteolytic enzymes which remove the outer coat of the virus, and convert potentially infectious virus (PIV) to infectious virus (IV). The intact double coat of potentially infectious reoviruses makes them exceptionally resistant to inactivation. Consequently, if they are inactivated, other viruses present in water are likely to be inactivated. (i) The potential importance of enzyme-enhanceable reoviruses as an indicator of viral pollution in water had never been assessed.

Conclusions

Madin-Darby bovine kidney (MDBK) cells, rhesus monkey kidney cells (LLC-MK_2) and human embryonic intestinal cells (Intestinal-407) were the most susceptible cell lines, respectively, for assaying reovirus serotypes 1, 2, and 3 previously isolated from sewage. The MDBK cells were the most sensitive when compared to LLC-MK_2 cells and Buffalo green monkey kidney (BGM) cells for assaying reoviruses that had been precipitated from sewage by protamine and had never been exposed previously to cells in culture.

The ICC method was found to be more sensitive, more specific and more rapid than the plaque assay procedure for assaying reoviruses isolated from sewage. The optimum inoculum size and centrifugation conditions were an inoculum of 0.25 mL centrifuged at 3000 rpm for 30 minutes in a Sorvall microtiter plate rotor.

When some protamine sewage precipitates were assayed for reoviruses, the potentially infectious viruses (PIV) were present in higher concentrations than the infectious viruses (IV). It appears that we encountered some chymotrypsin negative (CT^-) mutants in sewage in our study. The CT^- reovirus mutants are inactivated by chymotrypsin treatment. Some sewage appeared to convert PIV to IV. This would explain why it was not possible to enhance the infectivity of some reoviruses that had been extracted from sewage, i.e., the enhancement occurred while the virus was in the sewage.

During a one-year period, the concentration of reoviruses was compared with the concentration of enteroviruses in sewage. The reoviruses were consistently recovered in approximately 5-fold higher concentrations than the enteroviruses.

Recommendations

1. Reoviruses should be included when a standard test procedure for detecting viruses in water is adopted since this study and previous investigations have found that reoviruses are consistently present in sewage.

2. For recovery of reoviruses from water, additional studies need to be conducted to determine the best procedure. The procedure should be inexpensive and trouble free and capable of processing large volumes of water.

3. The immunofluorescent cell count and MDBK cells should be used in the assay of sewage for reoviruses.

4. The recommendation regarding enzyme treatment of reoviruses in water quality testing is that additional studies be conducted. The effect of adding EDTA directly to the sewage at the time of collection to protect any CT^- mutants from inactivation by enzymes in the sewage should be determined. This procedure, unfortunately, would inactivate rotaviruses.

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The complete report, entitled "Reoviruses in Water Pollution Testing," (Order No. PB 85-125 847; Cost: $10.00, subject to change) will be available only from:
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☆ U S GOVERNMENT PRINTING OFFICE, 1983 — 559-016/7874