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Concentration of Viruses in Faecal Samples from Patients with Gastroenteritis

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ABSTRACT

Faecal samples from 100 patients with gastroenteritis were examined by three methods; a) fluid absorption with polyacrylamide hydrogel, b) ammonium sulphate precipitation and c) ultracentrifugation to compare the concentration of virus particles before examination by negative stain electron microscopy. By all three methods, a similar content and range of viruses were detected, but the hydrogel technique presented some distinct advantages. Factors influencing fluid absorption from faecal extracts are outlined.

KEYWORDS

Viral gastroenteritis; faecal extracts; concentration of viruses; electron microscopy; polyacrylamide hydrogel; fluid absorption.

A number of virus species have been associated with viral gastroenteritis; of these rotaviruses constitute the major group. This disease affects predominantly the young of many animal species including man, and has a worldwide incidence (Holmes, 1979). In the acute phase of infection, virus particles may be excreted in very large numbers, often in excess of $10^8$ particles per g of faeces, and constitute a potential public health problem in effluent waters, in particular in developing countries. In spite of the excretion rate, many of these viruses are difficult to detect by in vitro procedures. They are unable to multiply in available types of cell culture. They are, however, readily detected by negative stain electron microscopy (EM) (Flewett, Bryden and Davies, 1974). A drawback to this technique is a need for the presence of approximately $10^6$ particles per g of faeces for detection. Therefore, to minimise negative findings in the early and late stages of infection, steps to increase the numbers of virus particles in stool preparations must be taken. Initial methods to improve EM sensitivity included, immune EM (Kapikian and others, 1972), density gradient formation (Kapikian and colleagues, 1973) and direct ultracentrifugation (Flewett and co-workers, 1974; Bishop and colleagues, 1974). Such techniques are difficult to perform and interpret, are laborious and may result in damage to virus ultrastructure. Ammonium sulphate precipitation (Caul, Ashley and Egglestone, 1978) and, more recently, the use of polyacrylamide hydrogel - lyphogel - (Whitby and Rodgers, 1980) have proved useful in concentrating virus particles in stools. The low-speed centrifugation technique reported by Narang and Codd (1979)
The 100 faecal specimens examined were prepared as 10% suspensions, clarified by low speed centrifugation and divided into three aliquots.

Sample 1. 0.55 ml was added to a vial containing 0.1 g lyphogel and left for four hours at room temperature or overnight at +4°C to allow absorption of fluids and salts. The residual volume was used for electron microscopy by negative staining.

Sample 2. 4 ml was added to a universal container holding 1.5 g ammonium sulphate, shaken well and left for a minimum of one hour at +4°C to encourage precipitation of proteins. The suspension was centrifuged at 10,000 g for 10 minutes and the pellet resuspended in distilled water for negative staining.

Sample 3. 5-10 ml was centrifuged at 8,000 rev/min for 45 minutes, the supernatant re-centrifuged at 40,000 rev/min for 60 minutes, and the pellet used for negative staining.

**TABLE 1 Comparison of Concentration Methods on 100 Faecal Specimens**

| Virus         | All 3 Methods | Positive by* | Ultra-centrifugation | Total |
|---------------|---------------|--------------|----------------------|-------|
|               | Lyphogel only | Ammonium Sulphate only | only |                  |
| Rotavirus     | 31            | 2            | 0                    | 0     | 33    |
| Adenovirus    | 6             | 1            | 1                    | 0     | 8     |
| Coronavirus   | 12            | 0            | 0                    | 0     | 12    |
| Calicivirus   | 2             | 0            | 0                    | 0     | 2     |
| Astrovirus†   | 1             | 1            | 0                    | 0     | 2     |
| tSRV          | 8             | 0            | 0                    | 1     | 9     |
| Total         | 60            | 4            | 1                    | 1     | 66    |

*Negative by all methods; †Small round isometric virus particles, 25 nm in diam.

**TABLE 2 Comparison of the Sensitivity of Concentration Methods**

| Virus          | Lymphogel Concentration (0.55 ml)* | Ammonium Sulphate Precipitation (4 ml)* | Ultra-centrifugation (0.55 ml)* (5 ml)* |
|----------------|------------------------------------|-----------------------------------------|----------------------------------------|
| Rotavirus      | 200 †                              | 100                                     | < 10                                   | 100 |
| Adenovirus     | 100                                | 100                                     | < 10                                   | 100 |
| Coronavirus    | 10                                 | 10                                      | < 10                                   | 10  |
| Calicivirus    | 100                                | 100                                     | < 10                                   | 100 |
| Astrovirus†    | 100                                | 200                                     | < 10                                   | 100 |
| tSRV           | 200                                | 200                                     | < 10                                   | 100 |

*Volume used for concentration
†Titres are reciprocal of highest dilution showing virus particles.

Of the 100 specimens, 60 were positive for virus by all three methods, and 3/4 negative. Additionally, four were positive by lyphogel only, one by ammonium sulphate only and one by ultra-centrifugation only (Table 1). A number of specimens positive for different virus types but in which the virus was present in minimal amount was concentrated as described, diluted 1/10, 1/50, 1/100, 1/200 and 1/400 in distilled water and examined for the presence of virus particles. Results were similar for each method provided appropriate concentration volumes were used (Table 2). The relative merits of each procedure are listed in Table 3, and illustrated in Fig. 1.
Concentration of Viruses in Faecal Samples

TABLE 3  Relative Advantages of each Concentration Method

|                              | Lyphogel | Ammonium Sulphate | Ultra- |
|------------------------------|----------|-------------------|-------|
|                              | Concentration | Precipitation    | centrifugation |
| Sensitivity/Reproducibility  | 1*       | 1                 | 1      |
| Laborious/Time Consuming in Preparation | 1         | 2                 | 3      |
| Expense                      | 2        | 1                 | 3      |
| Requirement for Equipment    | 1        | 2                 | 3      |
| Specimen Volume Needed       | 1        | 2                 | 3      |
| Distorts Virus Capsid        | 1        | 1                 | 2      |
| Salt Deposition Obscuring Virus Morphology | 1        | 2                 | 1      |
| Selects Dispersed Virus Populations | 1        | 1                 | 2      |
| Adaptable to Variable Specimen Numbers | 1        | 2                 | 3      |
| Safety Aspects               | 1        | 2                 | 3      |

* 1 = Fared best, 2 = Inbetween or variable, 3 = Fared worst.

Fig. 1. Virus particles from stools, negatively stained with 3% phosphotungstic acid, pH 6.5. Column 1 = rotaviruses, Column 2 = adenoviruses, Column 3 = coronaviruses. a,b, c = Lyphogel concentration; d,e = ammonium sulphate precipitation, note crystalline deposits obscuring virus morphology; f,g,h = ultracentrifugation, note capsid disruption, and removal of coronavirus projections. Bar = 100nm

The action of lyphogel, which was found to be temperature dependent (Fig. 2), was by physical absorption with ionic bonding of water, salts and small molecules of less than 20,000 daltons. Although the material expanded 6-fold on completion of fluid uptake, virus particles were not adsorbed onto the gel surface; antibody coated particles were unaffected and virus morphology was well preserved. Studies on laboratory prepared suspensions of echovirus and adenovirus showed that infectivity was increased by two log₁₀ dilutions after lyphogel concentration.
This procedure offers a simple, effective means of concentrating virus particles for morphological or infectivity studies on small specimen volumes. It can be readily introduced into any laboratory.

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