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Viruses in sewage as an indicator of their presence in the community

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SUMMARY

The results of a three year study of the viruses isolated from sewage by means of five tissue culture types are reported. The enteroviruses identified demonstrated a seasonal pattern which was similar to that of clinical isolates. Echoviruses and coxsackieviruses displayed a changing pattern of dominant serotypes in both sewage and clinical isolates; echovirus 6, 19, 3 and coxsackievirus B4, B5, A9 successively became the most common serotypes. The poliovirus in sewage was all vaccine-like in character. Reovirus, type 2 was abundant; adenovirus, of several serotypes, were the least often identified.

INTRODUCTION

Treated sewage is recognized as a valuable asset and is increasingly being recycled for drinking, recreation, industrial processing and agriculture. Recently, published recommendations by the World Health Organization suggested that the virus content of sewage should be monitored to determine how much and which viruses may be present and if they may constitute a health hazard (W.H.O. 1979). Viruses that can currently be isolated from sewage are the enteroviruses, adenoviruses and reoviruses. This information is also important for epidemiological purposes as the isolation of the viruses should reflect their prevalence within the community as a whole. A comparison with the results of virus isolations from patients who are ill, will determine their possible association with disease.

In Western countries an average of 150 g of faeces is passed each day by each member of the population. Sabin (1955) found that infected volunteers passed an average of 1×10^4 TCD_{50} of virus per g of faeces. Honig (1956) and Ramos-Alvarez (1954) found that 10% of healthy children under the age of 15 shed virus at any one time in comparable amounts to the volunteer study. Clarke (1962, 1964) suggested that these figures indicated that virus was present in sewage to the extent of 700–1000 TCD_{50}/100 ml. Kelly and Sanderson (1960) and Chin (1967) in studies on sewage samples suggested figures of 150 and 250 TCD_{50}/ml. The

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Thames Water Authority found 500 virus units/100 ml from sewage effluents in southern England (Slade, 1978). The gauze swab method of sampling (Moore, 1948) is not quantitative but the results should be representative of the total virus content of sewage. Dip sampling will indicate the number of viruses present at any given time.

Surveys of the viruses in sewage have been made in the United States (Horstmann, 1973; Bloom, 1959; Lamb, 1964), in Sweden (Böttiger, 1973) and in Israel (Fattal, 1977). One survey of reovirus in sewage was done in England (Craske, 1969). These authors reported a seasonal variation in isolation of the enteroviruses, the peak incidence being during summer and autumn. Reoviruses were found to be common all year; mention of adenovirus isolation is rare (Pittler, 1967). Changes in the prevalence and dominance of the enteroviruses, especially the echoviruses, was also noted. The studies of Fattal (1977) and Palfi (1971) in Hungary were designed preferentially to isolate polioviruses of which respectively 13 and 36% were found to be of wild type. They made comparisons between the results of virus isolation from sewage surveys and of faeces from normal population. It was found that sewage surveys provided the more meaningful results.

This paper examines the incidence of viruses in sewage in Reading, Great Britain, over a three year period, September 1974–September 1977 and compares this with the viruses isolated from clinically ill patients during the same time as reported to the Communicable Disease Surveillance Centre of the Public Health Laboratory Service.

MATERIALS AND METHODS

Swab collection and treatment

Cotton gauze swabs were regularly suspended for six-day periods in raw sewage and in the final treated effluent at a local sewage treatment works. The swabs were treated with 1 N-NaOH to produce a pH value of 9 and vigorously squeezed manually or mechanically to produce 15 ml of extract. The pH was returned to neutral with HCl, then a balanced salt solution was added. This mixture was placed in an ultrasonic waterbath for two minutes, followed by low speed centrifugation. The supernatant was mixed with ether and stored overnight at +4 °C. After the ether was removed, the extract from the swab was inoculated into each of five cell cultures and incubated for 21 days before being discarded as negative. Human amnion, human diploid fibroblasts, HEp_2, Vero and primary Rhesus monkey kidney cells were used to detect the presence of viruses (Sellwood, 1980).

Cultures which showed characteristics of virus cytopathic effect were passaged in cells of the same type. Passaged cultures were frozen at −20 °C before virus identification. Final identification was by means of serum neutralisation tests or haemagglutination inhibition tests for reovirus.

Dip samples

Five litre samples of water were taken from the River Thames and associated waters. Viruses were concentrated by a two stage method using membrane adsorption and protein precipitation (Slade, 1977). The viruses were isolated

Table 1. *Proportion* (%) *each virus group forms of total viruses isolated*

Total number of viruses isolated		Echo-virus	Polio-virus	Coxsackie-virus	Adeno-virus	Reo-virus
1974	105	44% (46)	4% (4)	11% (12)	8% (8)	33% (35)
1975	328	42% (137)*	14% (48)	11% (36)	6% (19)	27% (88)
1976	367	25% (93)	19% (70)	19% (70)	12% (45)	24% (89)
1977	226	36% (82)	11% (41)	18% (41)	16% (37)	19% (42)
1974–77	Total	34% (358)	16% (163)	15% (159)	11% (109)	24% (254)

using the BGM strain of African Green Monkey cells (Barron, 1970) suspended in agar. Plaques were removed and the viruses identified by means of serum neutralisation tests.

Communicable Disease Report

The Communicable Disease Surveillance Centre (CDSC) of the Public Health Laboratory Service collects reports of micro-organisms isolated from clinically ill patients from all parts of England and Wales. These results are tabulated and circulated to laboratories in the Communicable Disease Report. It was from this that the number of viruses isolated from patients quoted in this paper are derived. For epidemiological purposes Reading forms part of the Oxford Region.

RESULTS

Total isolates

Viruses were readily isolated from sewage, 487 swabs yielded over a thousand isolates. The proportion of swabs from which at least one virus was isolated was 94% for inlet and 66% for effluent samples at the beginning of the study. This increased to 100% and 91% respectively in 1977 because of improved methods and the abundance of one predominant echovirus serotype. The proportion of swabs that yielded two or more different virus types during the total period was 75% for inlet and 36% for effluent samples.

The echovirus group was the most numerous, adenoviruses were the least with the other enteroviruses and the reoviruses occupying intermediate positions. The echovirus group formed the greatest proportion of the total number of viruses isolated each year when one serotype predominated (Table 1). In 1974/5 echovirus 6 and in 1977 echovirus 3 were present in very large numbers; in 1976 a mixture of serotypes was isolated. The proportion of reoviruses found decreased throughout this study mainly because subsequent changes in technique favoured the quicker growing viruses.

Monthly variations in isolations

The number of enteroviruses and reoviruses isolated increased in the late summer and autumn (Fig. 1). Poliovirus isolations also followed this pattern despite an apparently regular vaccination programme. Echovirus isolations were numerous throughout the year of 1976. Adenovirus isolations were sporadic,

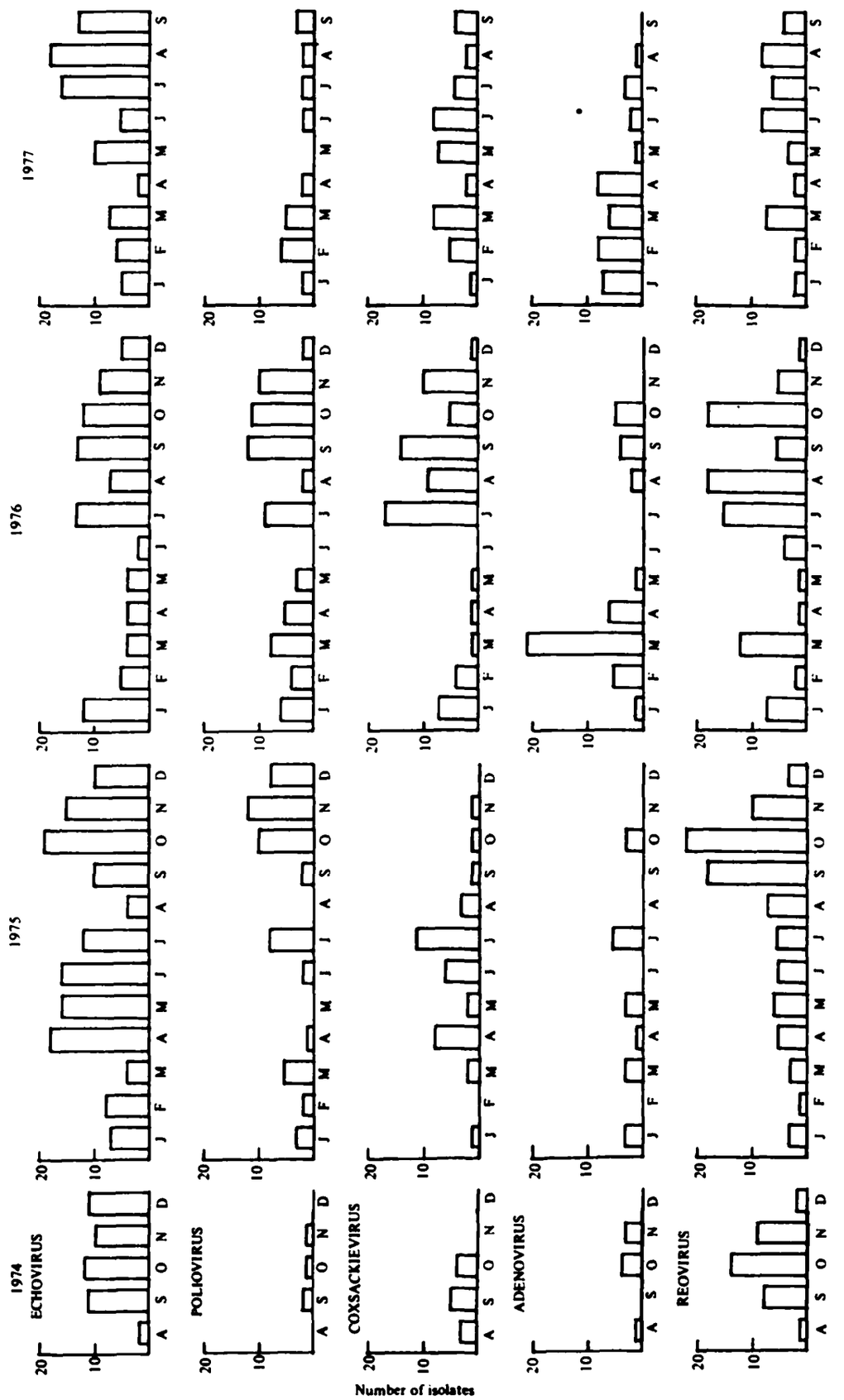

Fig. 1. Total number of viruses isolated from sewer swabs, Aug. 1974 to Sept. 1977.

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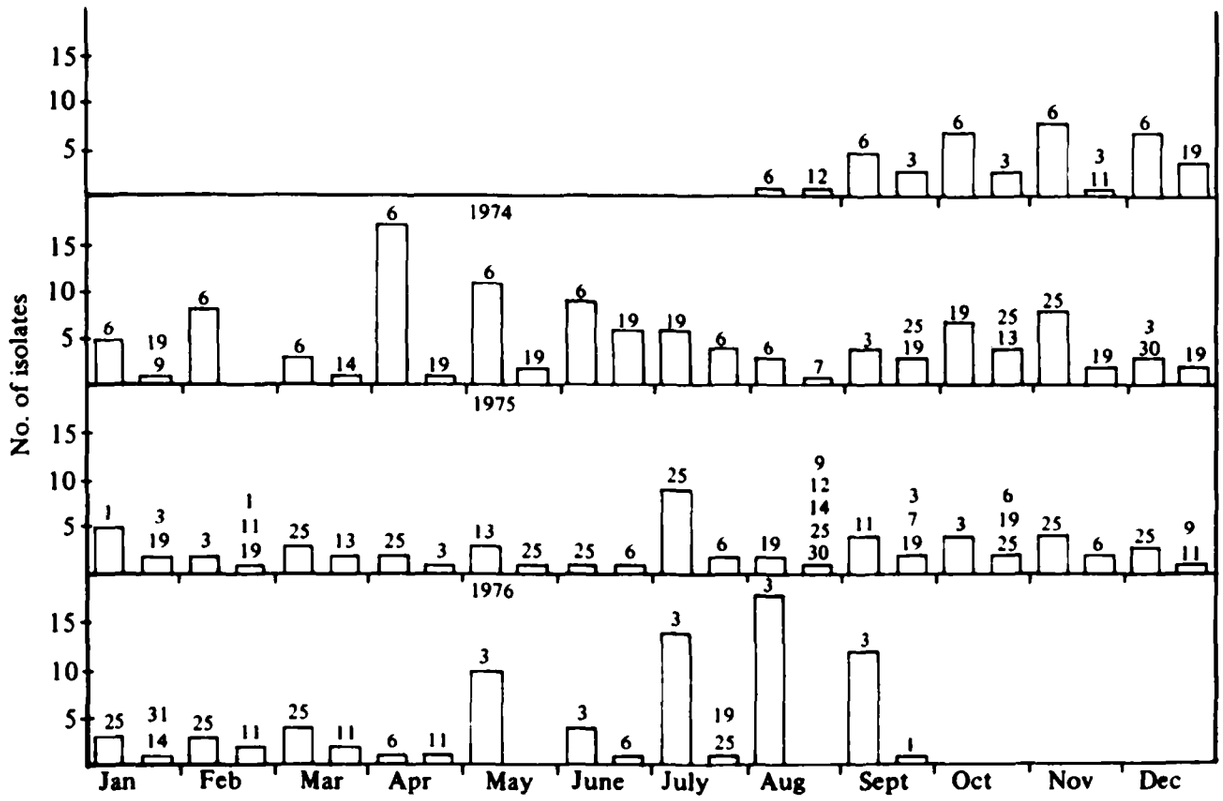

Fig. 2. Echovirus serotypes (denoted by figures above columns) isolated from sewer swabs in 1977.

though often more numerous in the early part of the year. This may have been affected by competition for cell culture by the faster growing echoviruses. Bacterial contamination of sewage extracts and cell culture affected the isolation rate in August 1975 and July 1976.

Echoviruses

The serotypes of echovirus from sewage (Fig. 2) were compared with the reports of echovirus isolations in the Communicable Disease Report (Table 2). A similar range of serotypes was reported to the CDR from the Oxford Region and on a national scale. From 1974 until mid-1975 the predominant serotype in clinical specimens was echovirus 19, while in the local sewage it was type 6. Later in 1975, type 19 was predominant in clinical and sewage specimens. From the end of 1975 type 19 and an increasing variety of serotypes were found in both types of specimens. After April 1977 echovirus 3 was very common in clinical specimens and overwhelmingly predominant in the sewage. Echovirus 25 was common in sewage but was not reported to the CDR.

Coxsackieviruses

The increased prevalence of coxsackievirus B5 during 1974 and of B4 during 1975 was common to both local sewage (Table 3) and clinical specimens (Table 4). Coxsackievirus A9 isolates were numerous by mid-1975 in clinical specimens rising to a maximum in the summer of 1976 when they were also isolated from sewer swabs. No major increase in B6 isolates was seen during 1976 or 1977 in clinical specimens though in sewage this serotype was numerous. Serotypes B1 and B5

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Table 2. *Echovirus isolations nationally and from the Oxford Region from patients reported to the CDR*

	Number of isolates (most common serotypes)																			
	National										Oxford Region									
Echovirus 1974	3	5	6	9	11	14	19	20	22	30	3	5	6	9	14	19	20	22	29	30
July-Sept.	—	—	125	55	—	—	333	—	—	—	—	—	3	—	—	10	—	—	—	—
Oct.-Dec.	—	—	135	—	—	—	213	—	44	—	—	—	3	—	—	5	—	—	—	—
1975																				
Jan.-Mar.	—	—	34	—	—	—	102	—	22	—	—	—	—	—	—	3	—	3	—	—
Apr.-June	—	—	29	—	—	—	258	—	8	—	—	—	1	—	—	1	—	—	—	—
July-Sept.	—	—	49	—	—	—	891	—	—	109	—	—	—	—	—	4	—	—	—	2
Oct.-Dec.	—	—	27	—	—	—	298	—	—	130	2	—	—	—	—	3	—	—	—	—
1976																				
Jan.-Mar.	—	—	—	—	—	18	42	—	—	48	—	—	—	—	1	—	1	—	1	1
Apr.-June	—	—	—	—	—	12	13	—	—	19	—	—	—	2	—	—	—	—	—	—
July-Sept.	—	46	—	98	—	—	—	—	—	172	—	10	—	7	—	—	—	—	—	—
Oct.-Dec.	—	50	—	52	—	—	—	—	—	47	—	4	—	2	—	—	—	—	—	—
1977																				
Jan.-Mar.	—	—	—	31	—	—	—	—	25	22	—	—	—	2	—	—	—	3	—	—
Apr.-June	—	—	—	82	17	—	—	—	22	—	5	—	—	7	—	—	—	—	—	—
July-Sept.	67	—	—	194	34	—	—	—	—	—	8	—	—	6	—	—	—	—	—	—

Table 3. *Coxsackievirus serotypes isolated from sewage* 1974–77

	Number of isolates																							
	1974						1975						1976						1977					
	B1	2	3	4	5	6	B1	2	3	4	5	6	A9	B1	2	3	4	5	6	B1	2	3	4	5	6
Jan.	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	2	1	—	3	—	1	—	—	—	—
Feb.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	—	—	—	—	3	1
Mar.	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	1	1	1	1	—	1	4
Apr.	—	—	—	—	—	—	—	—	—	1	3	4	—	—	—	—	—	—	1	—	—	—	—	—	2
May	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	1	—	—	2	—	2	3
June	—	—	—	—	—	—	—	—	1	5	—	—	—	—	—	—	—	—	—	—	—	—	—	1	6
July	—	—	—	—	—	—	—	—	—	11	—	—	4	4	—	2	—	3	4	1	—	—	—	3	—
Aug.	—	—	—	—	3	—	—	—	—	3	—	—	5	—	1	—	—	1	2	—	—	1	—	—	1
Sept.	—	—	1	—	4	—	—	—	—	—	1	—	—	—	—	5	—	3	6	—	—	1	—	2	1
Oct.	—	—	—	1	1	2	—	—	—	1	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—
Nov.	—	—	—	—	—	—	—	—	1	—	—	—	—	3	—	—	—	6	1	—	—	—	—	—	—
Dec.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—

were common in clinical isolates during the latter half of 1976 and throughout 1977 although B1 was found only sporadically in sewage. Coxsackievirus B2 was rarely isolated from sewage.

Serotypes of poliovirus, adenovirus and reovirus isolated from sewage

Poliovirus type 2 was always the most numerous of the three serotypes. Of the reoviruses, type 2 was predominant; the other two were difficult to identify with certainty. Those adenovirus serotypes grown were from a limited group of which 1, 2 and 5 were the most common.

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Table 4. *Coxsackievirus isolations from clinical specimens reported to CDR*

	Number of Isolates (most common serotypes)													
	National							Oxford Region						
	A9	B1	2	3	4	5	6	A9	B1	2	3	4	5	6
1974														
July-Sept.	—	—	106	—	—	133	—	—	—	2	—	—	4	—
Oct.-Dec.	—	—	105	—	—	50	—	—	—	2	1	—	—	—
1975														
Jan.-Mar.	—	—	39	—	28	—	—	—	—	—	—	—	—	—
Apr.-June	—	—	51	—	77	—	—	—	—	—	—	—	—	—
July-Sept.	95	—	—	—	196	—	—	1	—	—	—	1	—	—
Oct.-Dec.	65	—	—	—	57	—	—	6	—	1	—	—	—	—
1976														
Jan.-Mar.	48	—	—	—	26	—	—	1	1	—	—	—	—	—
Apr.-June	46	—	—	14	—	—	—	5	—	—	—	—	—	—
July-Sept.	162	—	—	—	—	100	—	8	2	—	—	—	—	—
Oct.-Dec.	—	88	—	—	—	111	—	—	2	—	—	—	1	—
1977														
Jan.-Mar.	—	57	—	—	—	57	—	—	1	—	—	—	—	—
Apr.-June	—	91	—	—	—	45	—	—	1	—	—	—	1	—
July-Sept.	—	184	—	—	—	89	—	—	6	—	—	—	1	—

Table 5. *Virus isolations from River Thames and associated waters*

	Number of occasions virus isolated								
	Coxsackievirus					Poliovirus			Echovirus
	B1	B2	B3	B4	B5	P1	P2	P3	E1
1974 (Sept.-Dec.)	—	—	—	2	15	—	8	—	1
1975	—	5	1	36	14	3	16	2	—
1976	11	4	6	15	70	4	22	6	—
1977	30	7	22	8	40	5	27	4	—

Characteristics of poliovirus isolates

Fifty poliovirus isolates were sent to Dr M. Roebuck at the Virus Reference Laboratory, Colindale for temperature marker tests. The viruses included all three serotypes and had been isolated in all the cell types from both raw sewage and effluent. All the isolates were found to have the growth characteristics of vaccine-like strains. Dextran inhibition tests were also done on the type 1 viruses and confirmed their vaccine-like character.

Isolates from the River Thames and associated waters

The coxsackieviruses isolated from the River Thames were found to correlate well with isolations from our local sewage and also from clinical reports (Table 5). Coxsackievirus B4 was prevalent in Thames water in 1975, then B5 and B1 became numerous in 1976 and 1977. All the poliovirus serotypes were found, type 2 being the most frequent isolate.

DISCUSSION

These data suggest that sewage can be an effective indicator for the presence of enterovirus, adenovirus and reovirus in the community in contrast with faeces surveys which have not been found to fulfill this role satisfactorily (Wilterdink, 1970; Horstmann, 1973). The study of less polluted river waters does not yield as much epidemiological information as does sewage but this may be a result of the different techniques employed. Further details of the environmental aspects of this study are to be published.

Although a changing pattern of serotype prevalence is seen with echoviruses and coxsackieviruses this is not so for poliovirus and reovirus. These results include obvious increases in the number of viruses isolated each summer and autumn and whenever a single echovirus serotype was predominant even though the sampling method employed was not strictly quantitative.

The serotypes from sewage were found to correlate well with those from clinical specimens but would not be reliable enough to predict which viruses would soon become important as a cause of clinical illness. Echovirus 14 and 11 were commonly found in patients and although found in sewage, their significance was not apparent. For echovirus 3, however, the sewage isolates predicted the increased importance of this serotype. Echovirus 25 was identified regularly from sewage but was not reported to the CDR which suggests that it may be less likely to cause disease than other serotypes. Echoviruses 5 and 9, although common in clinical specimens, were not isolated from sewage. This may reflect poor survival in the environment and in the latter's case, the overwhelming presence of type 3.

No major increase in coxsackievirus B6 isolates was seen during 1976 or 1977 in clinical specimens though in sewage this serotype was numerous.

The poliovirus isolates were all vaccine-like in character which probably reflects a high vaccine acceptance rate in this local area. Some natural infection and spread of these vaccine strains must also occur to account for the increase in isolates during the latter part of year. The origin of the reoviruses in sewage is not so clear. Large numbers were found but no human disease is known to be associated with their presence. Cattle also are infected but the number of isolates involved suggest that these isolates are likely to be of human origin.

The views expressed in this communication are the personal views of the authors.

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