J. Hyg., Camb. (1984), 93, 639–643
Printed in Great Britain

The detection of rotaviruses in products of wastewater treatment

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(*Received* 15 *May* 1984; *accepted* 23 *July* 1984)

SUMMARY

Rotaviruses present in products of wastewater treatment were assayed in MA104 cells by indirect immunofluorescence. Levels in settled sewage, activated sludge and effluent were greater than 10^3 per litre in March and April but virus was not detected during later months. This pattern correlated with the decline in laboratory reports of human rotavirus infection.

It is well known that rotaviruses and many other enteric viruses may be excreted in very large numbers (Banatvala, 1981) and are present, but greatly diluted, in wastewater (Selwood, Dadswell & Slade, 1981). Infectious virus survives well during wastewater treatment (Butler & Balluz, 1979) and a wide spectrum of types has been isolated from effluent (Irving & Smith, 1981) and sludge (Berg & Berman, 1980). One important practical problem in these studies is the relatively low concentrations of virus present, rarely more than 10^4/l, which means that detection by electron microscopy or by serological techniques may require difficult or time-consuming concentration methods. The presence of virus is therefore most satisfactorily demonstrated by detection of infectivity in cells. For human rotaviruses an indirect immunofluorescent test has been developed (Banatvala *et al.* 1975) and the results reported herein indicate the successful application of the method to selected samples of treated sewage.

Five litre samples of primary settled sewage and activated sludge effluent were tested as were 100 ml samples of a 24 h blend of raw sludge as well as single dip samples (100 ml) of activated sludge all from a sewage works with an industrial input of about 2%.

The procedure for the isolation of enteric viruses has been described elsewhere (Bates, Goddard & Butler, 1981) and is summarized in Fig. 1. The method for the selective isolation of rotavirus was based on that of England (1972). The suspended cell plaque test for enteroviruses in BGM cells was based on that described by Slade, Chisholm & Harris (1983). Indirect immunofluorescence for the assay of rotavirus in MA104 cells was based on the method described by Banatvala *et al.* (1975).

Rotaviruses were isolated in large numbers from various products of treated sewage on several occasions during early spring (Table 1), but not in later months even when concentrates from relatively large samples were prepared and when enteroviruses were readily detected (Table 2). There was no apparent correlation

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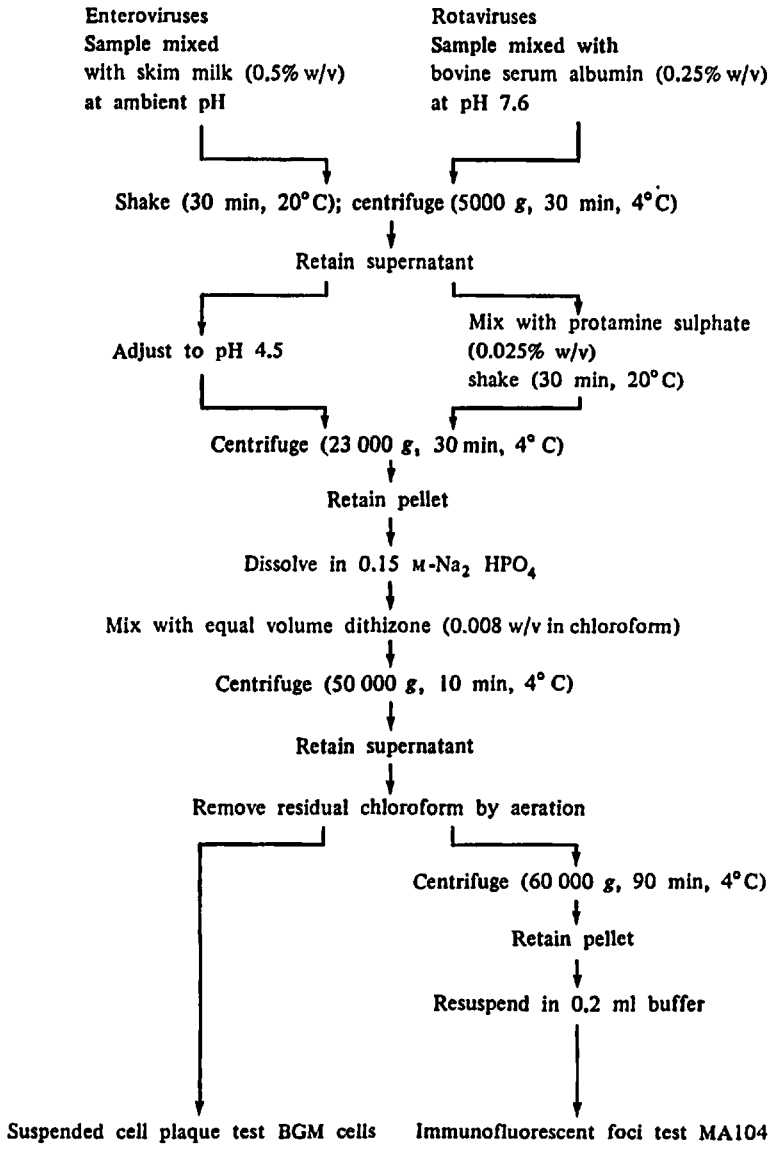

Fig. 1. Procedure for isolation of enteric viruses from products of wastewater treatment.

observed between the levels of enteroviruses in the samples with those of rotaviruses. However, there was a correlation between the failure to detect rotaviruses after April and the sharp decline in laboratory reports of human rotavirus infections notified by the Public Health Laboratory Service Communicable Disease Surveillance Centre for that period (private communication).

The apparent absence of rotaviruses in treated sewage products in early summer thus reflects on the likelihood that the amounts of virus excreted by the population had fallen to levels that were below the limits of our detection procedure. However, Steinman (1981) detected virus in summer in 2000-fold concentrates which he

https://doi.org/10.1017/S0022172400065219 Published online by Cambridge University Press

Table 1. *Human rotaviruses in products of sewage treatment*

Date	Sample	Volume examined (ml)	Titre (fluorescent foci per litre)
23 March	Settled sewage	200	8500
8 April	Settled sewage	200	940
	Activated sludge	100	3600
	Effluent	200	1500
29 April	Settled sewage	200	720
	Activated sludge	100	400
19 May	Settled sewage	200	N.D.
	Activated sludge	100	N.D.
	Effluent	200	N.D.
22 May	Settled sewage	1000	N.D.
8 June	Settled sewage	20000	N.D.
3 June	Raw sludge	20	N.D.
	Mesophilic digested sludge	40	N.D.
	Consolidated mesophilic digested sludge	40	N.D.
10 June	Raw sludge	200	N.D.
	Mesophilic digested sludge	200	N.D.
	Consolidated mesophilic digested sludge	200	N.D.
24 June	Raw sludge	100	N.D.
	Mesophilic digested sludge	200	N.D.
	Consolidated mesophilic digested sludge	200	N.D.

N.D. = None detected.

examined by both electron microscopy and enzyme-linked immunosorbent assay (ELISA). More recently, the ELISA technique has been successfully used to detect rotavirus in raw sewage (Goddard & Sellwood, 1983; Walter, Dobbenkau & Dunlop, 1983) and Hejkal, Smith & Gerba (1984) have examined the relative merits of indirect immunofluorescence and ELISA as well as the vagaries of sampling in relation to season and discussed effects on the incidence of enteric viruses in untreated sewage.

The ability to demonstrate rotavirus in wastewater products should provide an incentive to set up more extensive and thorough surveys of water and wastewater. If such tests were combined with an assay for hepatitis A virus, which can also be demonstrated by an indirect immunofluorescence technique (Kojima *et al.* 1981) as well as for other potentially pathogenic enteric viruses such as the Norwalk agent and similar viruses, we should go some way towards providing a more rational basis for the assessment of public health risks associated with viruses in the disposal of the products of wastewater treatment. At the moment there is little direct evidence for transmission of enteric virus infections as a result of the contamination of water or land by wastewater (Bolivar *et al.* 1978; Cabelli *et al.* 1982; Sutmoller *et al.* 1982)

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Table 2. *Human enteroviruses in products of sewage treatment*

Date	Sample	Volume examined (ml)	Titre plaque forming units per litre
3 March	Settled sewage	3000	N.D.
	Activated sludge	100	40
	Effluent	3000	N.D.
5 April	Settled sewage	3000	500
	Activated sludge	100	50
	Effluent	3000	2
4 May	Settled sewage	3000	43
	Activated sludge	100	1200
	Effluent	3000	1
2 June	Settled sewage	3000	700
	Activated sludge	100	200
	Effluent	3000	3
3 June	Raw sludge	20	31250
	Mesophilic digested sludge	40	1250
	Consolidated mesophilic digested sludge	40	750
10 June	Raw sludge	20	625
	Mesophilic digested sludge	40	313
	Consolidated mesophilic digested sludge	40	N.D.
24 June	Raw sludge	20	2000
	Mesophilic digested sludge	100	50
	Consolidated mesophilic digested sludge	20	500

N.D. = Not detected.

but it is well known that the consumption of uncooked sea foods harvested from contaminated water may well result in enteric disease (Gerba & Goyal, 1978; Caul & Appleton, 1982).

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