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VIRUSES IN THE AQUATIC ENVIRONMENT

ANGLIAN REGION NRA - EASTERN AREA

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Summary

The objects of this study were firstly to examine the viral content of water, sediment and shellfish samples taken from a range of sites encompassing sewage polluted saline, clean saline, clean freshwater, sewage affected freshwater and freshwater affected by discharges from meat processing plants. Secondly, to assess the suitability of specific enteric viruses, bacteriaphages (F+ coliphage and MS_2 phage), E. coli and faecal streptococci as indicator organisms for enteric viral contamination and finally to investigate the effectiveness of different sewage treatment methods in removing viruses.

1) *The number of viruses found in water, sediment and shellfish samples was low. Polluted and unpolluted sites showed no significant difference in virus numbers.*

2) *Only 2 types of enteric virus were found - coxsackievirus B4 and poliovirus 2. The presence of poliovirus in sewage is common due to the oral polio vaccination. Rota viruses were found at 2 sites only.*

3) *E. coli and faecal streptococci cannot serve as indicators for the presence of viruses, since viruses were absent in waters with high bacterial counts and present in waters with low bacterial counts.*

4) *Phage numbers were low, but were higher in waters with high bacterial counts, thus it is unlikely that bacteriaphages would be useful indicators of human virus contamination. Further work is required before any definite conclusions can be made.*

5) *Analysis for polio 2 and coxsackievirus B4 would be the most suitable means of environmental monitoring for human pathogenic viruses. This would prove extremely expensive though.*

6) *The extent of sewage treatment did not have a significant effect on the number of enteric viruses in the effluent. Thus it was concluded that sewage treatment is not effective in the removal of enteric viruses.*

7) *Bacteriaphages were removed by sewage treatment.*

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Introduction

More than 100 types of enteric viruses are excreted in human faeces and they represent a considerable potential health hazard in water used for potable supply, recreational activities and shellfish cultivation, especially as viruses survive the sewage treatment process well. Viral infections are caused either by entero-viruses which live and reproduce within the gastro-intestinal tract, eg. coxsackieviruses, echoviruses and polioviruses or by those which only occasionally infect the gastrointestinal tract such as rotaviruses, reoviruses, adenoviruses and the hepatitis A virus. Viruses are present in water in much lower numbers than bacteria and large volumes of water must be examined to detect them. It should be noted that the minimal infecting dose for bacteria is much higher than for viruses.

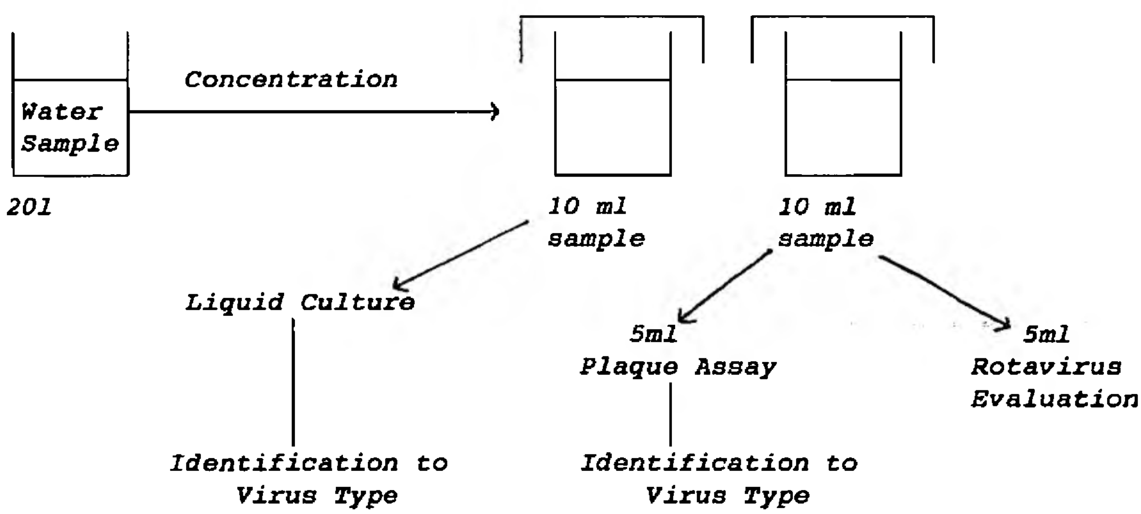
The methodology for detecting viruses is not yet suitable for routine laboratory tests since methods are both time consuming and expensive. Thus, if possible, reliable indicator organisms and analytical methods to serve as surrogates for the presence of viruses need to be found. Indicators may be drawn from bacterial, yeast and viral groups. Candidate bacterial indicators include the coliform group, faecal streptocci and clostridia species. Candidate viral indicators include either bacteriaphages (viruses which are parasitic on bacteria) or selected enteric viruses.

Enteric viruses readily attach themselves to solids in water and as a result tend to accumulate in sediments. Several species of shellfish rapidly accumulate viruses if they are present in the water. This is because shellfish filter feed and sieve out suspended food particles from a current of water passing through the shell cavity, bacteria and viruses are thus taken in along with food particles. Since the entire shellfish may be consumed raw or inadequately cooked, they can act as passive carriers of human pathogenic viruses. Viral diseases associated with contaminated shellfish consumption are infectious hepatitis and gastroenteritis.

Method

201 water samples and 1kg sediment samples were collected from a range of sites encompassing sewage polluted saline, clean saline, clean freshwater, sewage affected freshwater and freshwater affected by discharges from meat processing plants. Samples were sent to Wallace Evans for isolation and identification of enteric viruses. Wallace Evans capability statement was poliovirus types 1-3, echovirus types 1-35, coxsackievirus B1-6, A9, A16, adenovirus (all respiratory strains), reovirus types 1, 2 and 3, hepatitis A and rotavirus.

Wallace Evans experimental protocol was:-



F+ coliphage and MS_2 phage numbers were found. These are bacteriaphages specific to certain strains of <u>E. coli</u>. Water samples were also sent to the NRA laboratory at Kelvedon for <u>E. coli</u> and faecal streptococci analysis.

Oysters and/or mussels were collected from 7 sites, 4 sites being in sewage polluted saline waters and 3 in clean saline waters. 40g samples of flesh were analysed by Wallace Evans.

Sewage effluent samples were taken from various types of sewage treatment plant and analysed by Wallace Evans.

Results

Table I shows the numbers and types of virus found in the water, sediment and shellfish samples from the various sites. E. coli and faecal streps results for the water samples are also shown.

As can be seen, virus numbers were low. Only 2 types of enteric virus were found, these being polio 2 and coxsackievirus B4. Rotavirus were only found on two occasions, in the sediment from Northsea at Gunfleet Boat Club and in the shellfish from Butley Creek. The low numbers of virus found made it difficult to look at concentration of viruses in the sediment and shellfish. However, excluding zero counts, the average number of enteric viruses in the water column was 1.5/101, 5.9/kg in the sediments and 5/kg in the shellfish.

Phage numbers were low, the higher counts generally being found at sites with high bacterial counts.

Table II shows the number and types of viruses found in effluents from various types of treatment plant. As can be seen, the number of enteric viruses found in the effluents was very variable and extent of treatment does not appear to have had an effect on virus numbers.

A different case was found with the bacteriaphages and effluents receiving minimal treatment contained considerably more phages than those receiving secondary and/or tertiary treatment.

Discussion

(1) Viral content of water, sediment and shellfish samples

The results showed that virus numbers were low and no viruses were determined at the majority of sites. Perhaps larger samples should have been taken, although the handling of such large volumes/weights is problematicle. The time of year can effect virus numbers. Previous studies have shown a summer/early fall peak in virus numbers, and a study by Thames NRA on freshwater virology showed a peak in June/July. Lowest levels in the Thames NRA study were found in samples taken between December and January. This investigation was carried out mid-January to early February, so perhaps if the study was repeated in July, higher counts would be obtained. Two types of enteric virus were found during this investigation, coxsackievirus B4 and polio-2. It should be noted that with the advent of the oral polio vaccination, the predominant enteric viruses isolated from sewage have been the vaccine-derived polioviruses and these viruses may be found year round without a dominant summer/fall peak.

The low number of viruses found make it difficult to investigate viral concentration in sediments and shellfish. Viral contamination of shellfish was only found at one site, surprisingly at a clean site - Butley Creek. However, no enteric viruses were detected when shellfish from this site were re-examined in February 1992. It is interesting to note that polluted and unpolluted sites showed no significant difference in virus numbers. As has already been stated viruses readily attach to solids and accumulate in sediments. Adsorbed viruses remain infectious and survive longer than freely suspended viruses. Thus, a possible explanation is that the sediment resuspends in response to storms, movement of boats and swimmers, dredging and changes in water quality, viruses resuspended in the water column can then be transported from polluted to non-polluted waters used for swimming and shellfish harvesting.

(2) Indicator organisms

The low viral counts obtained did not aid this investigation. However, it appears unlikely that E. coli and faecal streptococci could be used as indicator organisms since in some cases no viruses were found at sites with very high bacterial counts, e.g River Yare at Brush Bend, Gorleston (no viruses, >50,000/100ml E. coli and >5000/100ml faecal streps), whilst in other cases viruses were found at sites with low bacterial counts, e.g Felixstowe Ferry (1 virus, 40/100ml E. coli and 22/100ml faecal streps). It is essential that an indicator organism survives in the aquatic environment for at least as long as the causative agents, and that a satisfactory relation can be established between the survivals of the respective organisms. There is evidence in the literature to suggest that viral pathogens may survive much longer than bacteria, thus it may be concluded that E. coli and faecal streps are unsuitable indicators of enteric viral pollution.

(2) Indicator organisms

Many workers have suggested that bacteriaphages may be suitable indicators of enteric viral contamination, since there is evidence that certain coliphages are as resistant as enteric viruses to stress in the environment. The results of this investigation did not reveal a significant relationship between phage and virus numbers, however, phage numbers did appear to relate to bacteria numbers. Further work is required before any conclusions can be drawn on the suitability of bacteriaphages as indicator organisms. The major advantage of using phages is that results can be obtained in 6 - 8 hours (Kenard and Valentine).

Enteric viruses themselves are the most meaningful, reliable and effective virus index for environmental monitoring, but methods are time consuming and expensive. The results obtained in this study would suggest that it is only wroth analysing for polio 2 and coxsackievirus B4.

(3) Virus removal by varying degrees of sewage treatment

The results showed that macerated only effluents contained as many or fewer enteric viruses than those receiving secondary and tertiary treatment. This indicates that sewage treatment is not effective in virus removal. This is not an entirely accurate comparison since sewage going to each plant was coming from different populations. A more accurate investigation should probably have compared the virus numbers in the raw sewage with numbers in the final effluent for each type of plant.

It does appear, however, that sewage treatment is effective for bacteriaphage removal, since macerated only effluents contained considerably more phages than treated effluents. This is possibly because bacteria are removed during treatment, and F+ coliphage and MS_2 phage are specific for particular strains of E. coli.

GLOSSARY

Bacteriaphage :	A virus parasitic on bacteria.
Enteric virus :	The term applied to any viruses disseminated by the faecal route.
Entero virus :	Viruses which live and reproduce within the gastro-intestinal tract.
Pathogen :	Any agent that can cause disease.
P F U :	Plaque forming unit. Plaques are colourless areas of dead cells surrounded by viable cells stained with neutral red, a vital dye. A plaque is produced by a single virion or an aggregate of virions on a monolayer culture of cells.
Primary settlement :	Stage in sewage treatment, following preliminary treatment, where suspended solids are settled out as sludge.
Secondary treatment :	Process in sewage treatment following primary settlement, involving the oxidation of dissolved and colloidal organic compounds in the presence of micro-organisms and other decomposer organisms.
Tertiary treatment :	Occasionally used after secondary treatment to produce a high quality effluent. There are various forms of tertiary treatment, e.g sand filters, grass plots and lagoons.

References

(1) *Kenard, R P, and Valentine, R.S. Rapid determination of the presence of enteric bacteria in water. Appl Microbiol, 1974, 27, 484-487.*

(2) *Rao and Melnick. Environment Virology.*

(3) *Sterritt, R M, and Lester, J.N. Microbiology for Environmental and Public Health Engineers.*

(4) *Thames NRA, Human viruses in the river Thames and tributaries.*

(5) *Water Research Centre technical report. TR67. Investigations of sewage discharges to some British coastal waters. April 1978.*

CLEAN SALINE WATERS

TABLE I

SITE & DATE (1991)	WATER						SEDIMENT				SHELLFISH			
	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
Felixstowe Ferry														
9/1	ND	ND			100	<10	ND	ND						
30/1	1U	ND	<1	<1	40	22	ND	ND	<1	0.5	ND	ND	1	<1
Butley Creek 16/1	ND	ND	<1	<1	40	25	ND	ND	5	<1	5P	5	<1	<1
6/2/92											ND			
Thorpness 23/1	ND	ND	<1	<1	150	150	ND	ND	<1	<1				
Dunwich 16/1	ND	ND	<1	<1	290	180								
Brancaster Straithe 16/1	1C	ND	1	<1	170	98	5U	ND	<1	<1	ND	ND	<1	<1

KEY

P = Polio 2 Virus
C = Coxsackievirus
U = Unidentified
ND = None detected

SEWAGE AFFECTED SALINE WATERS

SITE & DATE (1991)	WATER						SEDIMENT				SHELLFISH			
	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
Tidal Crouch above Burnham STW 23/1	ND	ND	<1	<1	7600	4100	5P	ND	<1	<1				
Tidal Blackwater @ Fullbridge 23/1	2P	ND	1	<1	3100	500	ND	ND	<1	<1				
Salcott Crk Quinces Corner 9/1	ND	ND			200	70	ND	ND			ND	ND		
West Mersea Hard 6/2	ND	ND	<1	<1	760	590	ND	ND	<1	<1				
West Mersea Pyfleet Creek 6/2											ND	ND	<1	<1
R Colne @ Rowhedge Ferry 9/1	4P	ND			41000	142000	5C	ND						
30/1	ND	ND	<1	1.5	1000	1300	ND	ND	<1	<1				
N Sea @ Gunfleet Boat Club 30/1	ND	ND	<1	<1	310	210	ND	ND	<1	1.5				
9/1	ND	ND			620	200	ND	5U						
Hamford Water @ Skippers Island 8/1	ND	ND			290		ND	ND			ND	ND		
Dovercourt - Warners Holiday Camp 9/1	ND	ND			<1000	100	5P	ND						
30/1	ND	ND	<1	<1	790	520	ND	ND	<1	0.66				

Cont'd ....

__SEWAGE AFFECTED SALINE WATERS Cont'd ...__

	WATER						SEDIMENT				SHELLFISH			
SITE & DATE (1991)	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
R Orwell - Fisons Quay 23/1	ND	ND	11	<1	14000	4300	ND	ND	1	<1				
R Orwell- Woolverstone Marina 23/1	1C	ND	6	<1	3100	1400	ND	ND	<1	<1	ND	ND	140	
Lowestoft North Beach 16/1	ND	ND	<1	<1	590	200	50	ND	<1	<1				
R Yare @ Brush Bend Gorleston 16/1	ND	ND			>50000	>5000								
Gt Yarmouth Power Station 16/1	ND	ND			600	550	ND	ND	<1	<1				
East Runton - near outfall 16/1	ND	ND	<1	<1	2600	1150	ND	ND	<1	<1				

CLEAN FRESHWATERS

SITE & DATE (1991)	WATER						SEDIMENT				SHELLFISH			
	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
R Waveney @ Ellingham Mill 16/1	ND	ND	<1	<1	2900	>2000	5C	ND	<1	<1				
R Glaven @ Thornage Br 16/1					2300	490	ND	ND	1	<1				
R Gipping @ Sproughton Intake 23/1	ND	ND	<1	<1	370	310	10P	ND	<1	<1				
6/2	ND	ND	<1	<1	2100	230	ND	ND	<1	<1				
Mill River A1093 Brightwell Rd Br 9/1	ND	ND			250	110	ND	ND						
30/1	ND	ND	<1	<1	250	64	ND	ND	<1	<1				
Alton Water SE Corner 9/1	ND	ND					ND	ND						
R Colne - E Mills Intake 23/1	1C	ND	<1	<1	170	120	5C	ND	<1	<1				
Cambridge Brk - Bures 23/1	ND	ND	<1	<1	150	130	ND	ND	<1	<1				

SEWAGE AFFECTED FRESHWATERS

	WATER						SEDIMENT				SHELLFISH			
SITE & DATE (1991)	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
Stebbing Brook u/s Felsted STW 23/1	1U	ND	<1	<1	110	70	5P	ND	<1	<1				
Stebbing Brook d/s Felsted STW 23/1	1P	ND	<1	<1	1900	1200	ND	ND	<1	<1				
R Wid u/s Shenfield STW 23/1	ND	ND	1	<1	3500	1200	ND	ND	<1	<1				
R Wid d/s Shenfield STW 23/1	ND	ND	<1	<1	6600	3800	ND	ND	<1	<1				
R Colne - Langley Mill 9/1 30/1	 ND 2U	 ND ND	 <1	 <1	 1070 2200	 570 1060	 ND ND	 ND ND	 <1	 2.33				
R Colne - Engaine Rd Br 9/1 30/1	 ND ND	 ND ND	 <1	 3	 2000 6300	 1500 3000	 ND ND	 ND ND	 <1	 <1				
Virley Brk @ Abbots Wick Farm 23/1	ND	ND	7	<1	6600	6600	ND	ND	<1	<1				
Virley Brk d/s Tiptree STW 23/1	2C	ND	<1	<1	4600	3300	ND	ND	22	<1				
Belstead Brook u/s Chantry STW 9/1 6/2	1P ND	ND ND	 <1	 <1	6600 2800	800 4800	ND 10P	ND ND	 <1	 <1				
Belstead Brook @ 9/1 Bourne Sluice 6/2	ND 	ND 	 <1	 <1	76000 7000	5600 3600	ND ND	ND ND	 <1	 <1				

Cont'd ...

SEWAGE AFFECTED FRESHWATERS cont'd ...

	WATER						SEDIMENT				SHELLFISH			
SITE & DATE (1991)	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
Above Dereham STW 16/1	ND	ND	<1	<1	400	102	ND	ND	<1	<1				
D/S Dereham STW 16/1	ND	ND	<1	<1	90000	>20000	ND	ND	<1	<1				
R Brett u/s Hadleigh STW 6/2	ND	ND	<1	<1	610	720	ND	ND	<1	<1				
R Brett d/s Hadleigh STW 6/2	ND	ND	<1	<1	5400	1800	5C	ND	<1	<1				

FRESHWATERS AFFECTED BY DISCHARGE FROM MEAT PROCESSORS

	WATER						SEDIMENT				SHELLFISH			
SITE & DATE (1991)	VIRUS		PHAGE		BACTERIA		VIRUS		PHAGE		VIRUS		PHAGE	
	ENTERO pfu/10l	ROTA ff/10l	F+ pfu/ml	MS_2 ff/ml	E COLI No/100ml	F STREPS No/100ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml	ENTERO pfu/kg	ROTA ff/kg	F+ pfu/ml	MS_2 FF/ml
Bernard Matthews Discharge 23/1	ND	ND	12	<1										
R Wang d/s discharge 23/1	10	ND	5	<1	11000	3600	ND	ND	<1	<1				

TABLE II

TABLE TO SHOW VIRUS NUMBERS & TYPES IN SEWAGE EFFLUENT FROM VARIOUS WORKS

STW	TYPE OF PLANT	DATE (91)	ENTEROVIRUSES		No ROTAVIRUS ff/100ml	PHAGES	
			No. pfu/100ml	Types		F+ pfu/ml	MS_2 FF/ml
Shenfield	Screening, Maceration Pasveer ditch (extended aeration) final settlement No tertiary	23/1	30	Polio 2 Coxsackievirus B4	ND	<1	<1
Halstead	Extended aeration No tertiary	9/1 30/1	10 ND	Coxsackievirus B4	ND ND	<1	6
Tiptree	Extended aeration No tertiary	23/1	ND		ND	6	<1
Chantry	Extended aeration No tertiary	9/1 6/2	ND 20	Polio 2	ND ND	<1	<1
Dereham	Biological Filters No tertiary	16/1	ND		ND	4	<1
Felsted	Biological Filters No tertiary	23/1	ND		ND	4	<1
Chelmsford & Witham	Aeration & biological filters. No tertiary	23/1	ND		ND	40	<1
Colchester	Biological filters & activated sludge No tertiary	9/1 30/1	40 10	Polio 2 + Coxsackievirus B4	ND ND	<1	24
Hadleigh	Biological filtration Tertiary=sand filter	6/2	10	Polio 2	ND	<1	<1

Cont'd ...

TABLE II

Cont'd ...

TABLE TO SHOW VIRUS NUMBERS & TYPES IN SEWAGE EFFLUENT FROM VARIOUS WORKS

STW	TYPE OF PLANT	DATE (91)	ENTEROVIRUSES		No ROTAVIRUS ff/100ml	PHAGES	
			No. pfu/100ml	Types		F+ pfu/ml	MS_2 FF/ml
Gt Cornard	Biological filters Tertiary = Lagoons	6/2	Inflow to lagoon:ND	Polio 2	ND	2	<1
			Discharge from lagoon: 10		ND	1	<1
Cliff Quay	Primary settlement only	23/1	ND		ND	>200	<1
Clacton	Maceration only	9/1 30/1	20 ND	Polio 2	ND ND	<1	>200
Felixstowe	Maceration only	23/1	ND		ND	>200	<1
Dovercourt	Maceration only	9/1 30/1	30 ND	Polio 2	ND ND	<1	>200