Evaluation of coliphages as indicators of the virological quality of sewage-polluted water

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Abstract

Coliphage counts obtained by plaque assays using *Escherichia coli* strains C603, K12 *Hfr* or B as hosts at 37 °C or 25 °C, were compared in tests on waters of different quality and origin. Strain C603 at 37 °C yielded the highest counts, and was used in assays to compare numbers of coliphages with those of enteric viruses, standard plate counts, total and faecal coliform bacteria, faecal streptococci and acid-fast bacteria in wastewater, river and dam water, and treated drinking-water supplies. Coliphages generally outnumbered enteric viruses by a factor of 1 000 or more. Ratios of average counts of coliphages to those of other organisms tended to fluctuate, but showed that coliphage counts could give a useful estimate of numbers of other micro-organisms in sewage-polluted water. Evidence is presented that, even though counts of coliphages may not always directly correlate with those of enteric viruses, coliphages meet the basic requirements of an indicator for the virological safety of water, and that coliphage assays in combination with the standard plate count and counts of coliform and acid-fast bacteria, offer a practical and reliable indicator system for evaluating the virological safety of treated drinking-water supplies, even in the case of drinking-water directly reclaimed from wastewater.

Introduction

Currently available virological technology has the following serious shortcomings for water quality evaluation: Practical methods for the detection of viruses of primary concern such as the hepatitis A virus and gastroenteritis viruses including the rota- and norwalk viruses are not available, and techniques for both the recovery and identification of human enteric viruses have limited sensitivity, are time-consuming and expensive, and require highly skilled labour and sophisticated laboratory facilities (IAWPRC Study Group on Water Virology, 1983). In view of these shortcomings, the evaluation of the virological quality of water heavily depends upon the use of indicator organisms. The basic requirements of indicators are that they should be present in water environments whenever enteric viruses are present, they should be at least as resistant to water purification and disinfection processes as enteric viruses, and they should be detectable by simple, practical, reliable, rapid and economical techniques (Berg, 1978).

Various bacterial indicator systems including coliform bacteria, the standard plate count, faecal streptococci and acid-fast bacteria proved useful for assessing the virological quality or safety of a wide variety of waters (Grabow *et al.*, 1980; Cabelli *et al.*, 1982; IAWPRC Study Group on Water Virology, 1983). However, in view of the fundamental differences between bacteria and viruses, indicators more closely related to enteric viruses would be preferable for the evaluation of virological water quality, and particularly the behaviour of viruses in water treatment processes (Berg, 1978). Bacteriophages may play a valuable role in this regard because their structure, composition, morphology and size closely resemble that of enteric viruses, basically the most important difference being that bacteriophages are viruses which have bacteria as hosts while enteric viruses are viruses which have mammalian cells as hosts (Grabow *et al.*, 1980; IAWPRC Study Group on Water Virology, 1983).

This paper deals with an evaluation of coliphages (bacteriophages which infect strains of *Escherichia coli*) as indicators of the virological quality of water. The suitability of three host strains and incubation of plaque assays at two different temperatures were compared in order to optimize coliphage detection methods. The best method was then evaluated as indicator of the virological quality of water by comparing counts of coliphages in various raw and treated waters with those of enteric viruses and commonly used bacterial indicators.

Materials and Methods

Water samples. Grab samples were collected in sterile nalgene bottles containing an adequate amount of a sterile sodium thiosulphate solution to neutralize residual chlorine in the water samples (Grabow and Du Preez, 1979). Samples analysed within 4 h were not cooled, but samples kept for 4 to 24 h were cooled to at least 10 °C, but never frozen. Samples were homogenized (Grabow and Du Preez, 1979) prior to analysis. Samples of settled sewage, activated sludge effluent and Apies River water downstream of the discharge of purified wastewater, were collected at the Daspoort purification works in Pretoria (Grabow and Du Preez, 1979). Samples taken from the Vaal River, its barrage, and Klip and Suikerbosrand River tributaries, as well as from raw water intake points and treated water distribution lines of the Rand Water Board, have been described (Hattingh, 1977; Rand Water Board, 1980, 1981). Details on waters sampled in Windhoek have been published (Grabow, 1977; Grabow *et al.*, 1978, 1981, 1982b); the maturation pond effluent (WR1) was the raw water intake to the reclamation plant, the Goreangab Dam water (WD22) the raw water intake for conventionally treated drinking-water (WD23), and the drinking-water tested consisted only of reclaimed water (WR14), conventionally treated Goreangab Dam water (WD23), or a mixture of the two (WR14 + WD23).

Counts of coliphages. A modified double-layer-agar method (Adams, 1959) was used for plaque assays. The bottom layer contained 11 g Difco agar, 13 g Difco Bacto tryptone, 8 g sodium chloride and 1,5 g glucose per 1 ℓ distilled water. The top layer contained 6 g agar, 10 g tryptone, 8 g sodium chloride and 3 g glucose per 1 ℓ water. The bottom layer (15 mℓ) in 90 mm diameter petri dishes was covered with a layer containing 2,5 mℓ top layer medium, 0,2 mℓ of a 37 °C Difco nutrient broth culture of the host and 1,0 mℓ test sample. Plaques were counted after incubation for 16 h at 37 °C or 25 °C. Samples of 10 mℓ were analysed by plating 10 petri dishes with 1 mℓ each of undiluted test sample. High counts were titrated by plating saline dilutions in triplicate. Host strains *E. coli* B and K12 *Hfr* (Kott *et al.*, 1978) were supplied by Prof. Y. Kott (Technion, Haifa, Israel) and

strain C603 (Logan *et al.*, 1981) by Prof. S.B. Primrose (University of Warwick, Coventry, England). Strain C603 grows relatively slowly in unsupplemented broth because it is a histidine and arginine auxotroph. Cultures of this strain were therefore incubated for 48 h instead of 24 h as in the case of prototrophic hosts.

Counts of bacteria. Standard plate counts were determined by means of pour plate cultures using Biolab yeast extract agar and incubation for 48 h at 35 °C (SABS, 1971). All other bacteria were counted by membrane filtration, using Difco m-Endo agar LES and incubation for 24 h at 35 °C for total coliform bacteria (Grabow and Du Preez, 1979), Biolab M-FC agar without rosolic

TABLE 1
COUNTS OF COLIPHAGES USING THREE HOSTS AND TWO INCUBATION TEMPERATURES FOR PLAQUE ASSAYS ON SELECTED WATERS

Determinants	Count/10 mℓ					
	E. coli K12 *Hfr*		*E. coli* B		*E. coli* C603	
	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C
Daspoort settled sewage						
27 March 1981 to 14 May 1981						
No. of tests	13	13	13	13	13	13
No. of positive tests	13	13	13	13	13	13
Average count	$18,5 \times 10^4$	$17,2 \times 10^4$	$16,3 \times 10^4$	$13,3 \times 10^4$	$43,6 \times 10^4$	$38,4 \times 10^4$
Median count	18×10^4	17×10^4	14×10^4	12×10^4	39×10^4	29×10^4
Range of counts	$(7\text{-}30) \times 10^4$	$(10\text{-}27) \times 10^4$	$(10\text{-}29) \times 10^4$	$(8\text{-}21) \times 10^4$	$(21\text{-}133) \times 10^4$	$(18\text{-}134) \times 10^4$
Standard deviation	$5,1 \times 10^4$	$4,4 \times 10^4$	$5,6 \times 10^4$	$4,3 \times 10^4$	$28,5 \times 10^4$	$30,3 \times 10^4$
Highest count of all	0	0	0	0	11	2
Highest count for host	8*	4*	12	1	10	3
Daspoort activated sludge effluent						
15 Jan. 1981 to 6 Feb. 1981						
No. of tests	13	13	13	13	13	13
No. of positive tests	11	12	10	10	13	13
Average count	86	129	115	113	406	315
Median count	6	8	4	8	46	40
Range of counts	0-374	0-516	0-592	0-508	2-1980	8-1400
Standard deviation	135,5	183,1	189,6	163,4	646,7	469,0
Highest count of all	0	0	0	0	8	5
Highest count for host	0*	12*	5*	6*	8	5
Apies River water						
9 Feb. 1981 to 26 March 1981						
No. of tests	12	12	12	12	12	12
No. of positive tests	12	12	12	12	12	12
Average count	2463	2548	2756	2626	4031	3467
Median count	1720	1750	2080	1860	2810	2570
Range of counts	328-7160	538-5760	626-6100	636-6280	2060-7880	1300-7540
Standard deviation	1921,8	1757,7	2050,1	1998,3	2242,0	2197,9
Highest count of all	0	0	0	1	10	1
Highest count for host	3	9	5	7	11	1
Vaal River, RWB intake at Zuikerbosch						
7 May 1981 to 8 June 1981						
No. of tests	4	4	4	4	4	4
No. of positive tests	4	4	4	4	4	4
Average count	48	47	27	25	78	75
Median count	40	51	24	22	84	68
Range of counts	32-78	24-62	8-52	12-42	30-112	40-122
Standard deviation	21,3	16,7	18,9	13,5	38,0	34,5
Highest count of all	0	0	0	0	1*	2*
Highest count for host	2	2	2	2	1*	2*
Vaal Barrage, RWB intake Vereeniging No. 2						
29 Jan. to 4 March 1981						
No. of tests	5	5	5	5	5	5
No. of positive tests	5	5	5	5	5	5
Average count	84	124	131	144	241	226
Median count	40	54	114	130	164	156
Range of counts	20-244	44-344	18-314	52-338	20-740	26-650
Standard deviation	91,7	127,1	110,6	115,2	286,3	244,6
Highest count of all	0	0	0	1	2	2
Highest count for host	0	5	1	4	2*	2*

RWB = Rand Water Board
*Where total of highest counts do not equal total number of tests, one or more counts were equal for both tests temperatures

acid and incubation for 24 h at 44,5 °C for faecal coliform bacteria (Grabow *et al.*, 1981), Difco M. Enterococcus agar and incubation for 48 h at 44,5 °C for faecal streptococci (Grabow and Nupen, 1972), and an enriched Middlebrook 7H9 agar medium and incubation for 7 days at 35 °C for acid-fast bacteria (Grabow *et al.*, 1980).

Enumeration of enteric viruses. Settled sewage contained enough viruses for direct titration. Prior to inoculation of cell cultures, samples were treated with chloroform to inactivate organisms other than viruses. This was done by shaking up 3 mℓ chloroform with 20 mℓ sample, allowing the chloroform to settle overnight at 4 °C, and centrifugating (2 500 x g for 30 min), after which the supernatant was removed and used for titration. The other waters contained too few viruses for direct titration and viruses were recovered from 10 ℓ samples by means of pressure ultrafiltration using 150 mm diameter type XM50 Amicon membranes and high-flow stirred cell units (Nupen *et al.*, 1980; Grabow *et al.*, 1982b). Viruses in chloroform-treated samples or concentrates were enumerated by $TCID_{50}$ evaluation using eight cell culture tubes per dilution, or presence-absence tests by inoculating the entire concentrate of a sample into a 1 ℓ Roux cell culture flask (Nupen *et al.*, 1980; Grabow and Nupen, 1981). Primary vervet kidney cells were grown in Eagle minimal essential medium with Earle's salts (EMEM, Flow Laboratories) supplemented with antibiotics and foetal calf serum (Grabow *et al.*, 1982a), and read daily for cytopathogenic effects for three weeks (Nupen *et al.*, 1980; Grabow and Nupen, 1981). When necessary virus isolates were identified by the National Institute for Virology, Johannesburg, using conventional serological techniques.

Results

Details of an evaluation of coliphage counts obtained by using different hosts and incubation temperatures in plaque assays on waters of different quality and origin, are presented in Tables 1 and 2. These results show that in assays using *E. Coli* K12 *Hfr* as host, higher average phage counts were obtained when plates were incubated at 37 °C in tests on Daspoort settled sewage, but in tests on all the other waters average counts tended to be higher when plates were incubated at 25 °C. When *E. coli* B was used as host in tests on the same water samples, incubation at 37 °C yielded slightly higher average counts for all waters except Apies River water. Differences in counts for both *E. coli* K12 *Hfr* and *E. coli* B incubated at 37 °C or 25 °C were marginal as is illustrated by the lack of correlation between average and median counts and the number of tests for which each incubation temperature yielded the highest count. Host strain *E. coli* C603 behaved differently in so far that incubation at 37 °C consistently yielded higher average and median counts than at 25 °C, although the difference was marginal for samples from both Vaal River stations. An outstanding feature of the evaluation of host strains was that *E. coli* C603 consistently yielded higher coliphage counts than the other two hosts. In tests on only two samples, one of Vaal Barrage water and one of Apies River water, *E. coli* B yielded slightly higher counts than *E. coli* C 603.

Assessment by Student's t-test of results of the evaluation of counts obtained by using different hosts and incubation temperatures, shows that in terms of a 95 % confidence limit the difference in counts derived by incubation at 37 °C or 25 °C was not significant for all three hosts (Table 2). However, *E. coli* C603

TABLE 2
STUDENT'S t-TEST ASSESSMENT OF SELECTED COLIPHAGE COUNT IN TABLE 1

Water, *E. coli* hosts and assays	t-test	Limit*	Assessment
Daspoort settled sewage			
K12 *Hfr*: 37 °C vs 25 °C	0,68	1,71	Difference not significant
B: 37 °C vs 25 °C	1,47	1,71	Difference not significant
C603: 37 °C vs 25 °C	0,43	1,71	Difference not significant
C603/37 °C vs K12 *Hfr*/37 °C	3,00	1,71	C603/37 °C significantly higher
Daspoort activated sludge effluent			
K12 *Hfr*: 37 °C vs 25 °C	0,66	1,71	Difference not significant
B: 37 °C vs 25 °C	0,03	1,71	Difference not significant
C603: 37 °C vs 25 °C	0,40	1,71	Difference not significant
C603/37 °C vs K12 *Hfr*/25 °C	1,43	1,71	Difference not significant
Apies River water			
K12 *Hfr*: 37 °C vs 25 °C	0,11	1,72	Difference not significant
B: 37 °C vs 25 °C	0,15	1,72	Difference not significant
C603: 37 °C vs 25 °C	0,57	1,72	Difference not significant
C603/37 °C vs K12 *Hfr*/25 °C	1,73	1,72	C603/37 °C significantly higher
Vaal River/Zuikerbosch			
K12 *Hfr*: 37 °C vs 25 °C	0,06	1,94	Difference not significant
B: 37 °C vs 25 °C	0,15	1,94	Difference not significant
C603: 37 °C vs 25 °C	0,10	1,94	Difference not significant
C603/37 °C vs K12 *Hfr*/37 °C	1,19	1,94	Difference not significant
Vaal Barrage/Vereeniging intake			
K12 *Hfr*: 37 °C vs 25 °C	0,51	1,86	Difference not significant
B: 37 °C vs 25 °C	0,16	1,86	Difference not significant
C603: 37 °C vs 25 °C	0,08	1,86	Difference not significant
C603/37 °C vs B/25 °C	1,63	1,86	Difference not significant

*95% Confidence t-Test limit for degrees of freedom concerned.

and incubation at 37 °C yielded significantly higher counts than the alternative hosts at any incubation temperature for samples of Daspoort settled sewage and Apies River water. Differences in counts obtained with *E. coli* K12 *Hfr* and *E. coli* B were not sufficient. The superiority of *E. coli* C603 as a host for detecting the highest numbers of coliphages is also illustrated by the finding that in the Daspoort activated sludge effluent which contained relatively low numbers of phages, plaque assays using *E. coli* strains K12 *Hfr* or B occasionally yielded negative results while assays using strain C603 as host yielded positive results for all samples.

Since plaque assays using *E. coli* C603 as host and incubation at 37 °C generally yielded the highest coliphage counts, they were applied to compare the numbers of coliphages in a variety of

TABLE 3
COUNTS OF MICRO-ORGANISMS IN WATER SAMPLES OF THE VAAL DAM, VAAL RIVER AND TRIBUTARIES

Determinants	Plate Count (c/1 mℓ)	Total Coliforms (c/100 mℓ)	Faecal Coliforms (c/100 mℓ)	Acid-fast Bacteria (c/100 mℓ)	Faecal Strepto. (c/100 mℓ)	Enteric Viruses (c/10 ℓ)	Coli-phages (c/10 mℓ)
Vaal Dam, RWB intake (A7)							
24 April 1981 to 8 March 1983							
No. of test	34	34	34	22	34	34	34
No. of positive tests	34	34	33	22	30	0	15
Average count	489	108	19	17	7	0	33
Median count	109	20	7	4	3	0	0
Range of counts	13-4000	1-1290	0-210	1-80	0-82	0	0-981
Standard deviation	938,8	251,9	40,9	23,6	15,0	0	167,8
Ratio of average: phage/org.	$6,7 \times 10^{-3}$	3,1	17,4	19,4	47,1	$>33 \times 10^{3}$	
Vaal River, RWB intake (ZRW)							
7 April 1981 to 10 May 1983							
No. of tests	46	46	46	46	46	46	46
No. of positive tests	46	46	44	40	41	0	42
Average count	2900	264	21	116	8	0	25
Median count	600	53	9	27	5	0	7
Range of counts	$(1\text{-}276) \times 10^{2}$	5-3000	0-90	0-1400	0-30	0	0-234
Standard deviation	6150	579,2	24,1	279,8	8,3	0	42,5
Ratio of average: Phage/org.	$8,6 \times 10^{-4}$	1,0	11,9	2,2	31,3	$>25 \times 10^{3}$	
Suikerbosrand River (S2)							
13 Oct. 1981 to 15 March 1983							
No. of tests	28	28	28	25	28	28	28
No. of positive tests	28	28	28	25	28	0	28
Average count	13×10^{3}	448	136	17	52	0	55
Median count	$2,3 \times 10^{3}$	163	34	13	32	0	25
Range of counts	$(3\text{-}747) \times 10^{2}$	17-5300	7-1100	1-63	3-370	0	0-370
Standard deviation	$20,7 \times 10^{3}$	1033,4	268,3	16,7	69,9	0	75,1
Ratio of average: phage/org.	$4,2 \times 10^{-4}$	1,2	4,0	32,4	10,6	$>55 \times 10^{3}$	
Klip River (K19)							
21 April 1981 to 17 May 1983							
No. of tests	43	43	43	33	43	43	43
No. of positive tests	43	43	43	31	43	20	43
Average count	215×10^{2}	257×10^{2}	16×10^{2}	230	247	546	488
Median count	86×10^{2}	180×10^{2}	7×10^{2}	77	101	0	234
Range of counts	$(5\text{-}2100) \times 10^{2}$	$(16\text{-}790) \times 10^{2}$	$(1\text{-}74) \times 10^{2}$	0-1930	29-3760	0-6300	36-3480
Standard deviation	325×10^{2}	315×10^{2}	17×10^{2}	408,9	568,5	1402,5	587,0
Ratio of average: phage/org.	$2,3 \times 10^{-3}$	0,19	3,05	21,2	19,8	893,8	
Vaal Barrage, RWB intake (A3)							
6 Oct. 1981 to 17 May 1983							
No. of tests	38	38	38	32	38	38	38
No. of positive tests	38	38	38	29	38	14	38
Average count	4900	2271	160	311	24	89	145
Median count	4100	660	69	34	15	0	58
Range of counts	$(10\text{-}170) \times 10^{2}$	$(28\text{-}2) \times 10^{4}$	1-1620	0-2000	1-166	0-928	1-1563
Standard deviation	3480	3938	285,0	539,6	30,0	193,9	271,1
Ratio of average: phage/org.	3×10^{-3}	0,6	9,1	4,7	60,4	$1,6 \times 10^{3}$	
Vaal Barrage, RWB intake (A9)							
7 April 1981 to 26 April 1983							
No. of tests	46	46	46	46	46	46	46
No. of positive tests	46	42	39	37	40	3	37
Average count	2530	181	35	66	32	4	7
Median count	2600	49	11	15	8	0	3
Range of counts	$(0,6\text{-}100) \times 10^{2}$	0-4300	0-547	0-530	0-1003	0-93	0-54
Standard deviation	2170	633,8	100,9	119,2	147	16,8	10,1
Ratio of average: phage/org.	$2,8 \times 10^{-4}$	0,4	2,0	1,1	2,2	$1,8 \times 10^{3}$	

water environments to those of enteric viruses and selected bacteria used as indicators of water quality. In water of the Vaal Dam as well as the Vaal and Suikerbosrand Rivers, average counts of coliphages were outnumbered only by those of the standard plate count (see ratios of average counts of coliphages to those of other organisms in Table 3). However, in water of the Klip River, and the A3 and A9 samples of the Vaal River Barrage, average counts of total coliform bacteria were also higher than those of coliphages. Windhoek settled sewage, maturation pond effluent and Goreangab Dam water similarly had higher average standard plate and coliform counts than coliphage counts, but in addition, the settled sewage and Goreangab Dam water also had higher average counts of faecal coliforms, and in the case of Goreangab Dam water even the average count of faecal streptococci was higher than that of coliphages (Table 4).

Coliphages were not detected in any of 45 samples of Windhoek drinking-water, while all the samples yielded positive plate counts, two contained coliform bacteria, and an enteric virus was recovered from one (Table 4). Rand Water Board drinking-water also had positive plate counts for all samples tested, while one of the 146 samples was positive for total coliforms, 88 for acid-fast bacteria (which were not included in tests on Windhoek drinking-water) and six for coliphages (Table 5).

In waters from which coliphages were isolated, on average the coliphages were outnumbered by a factor 1:1 000 by the standard plate counts. In samples showing both coliphages and enteric viruses, the latter were generally outnumbered by a factor 1 000:1 by the coliphages. The much higher incidence of coliphages compared to enteric viruses in the water tested, is also illustrated by the fact that 211 of all the samples analysed yielded positive results for coliphages and negative results for enteric viruses, while only two samples (one Vaal River Barrage A9 and one Windhoek drinking-water sample) were positive for enteric viruses and negative for coliphages.

No attempt was made to characterize coliphages recovered in plaque assays using *E. coli* C603 as host and incubation at 37 °C. However, one plaque representing a plaque morphology which was very common in these assays, was isolated and the phage investigated in some detail. The phage was recovered from a sample of Vaal River Barrage (A3) water and designated V1 (V for Vaal River). It produced relatively large, lytic plaques with a diameter of 5 to 10 mm (Figure 1a) on *E. coli* C603 but did not plaque on

TABLE 4
COUNTS OF MICRO-ORGANISMS IN SELECTED WINDHOEK WATERS

Determinants	Plate Count (c/1 mℓ)	Total Coliforms (c/100 mℓ)	Faecal Coliforms (c/100 mℓ)	Faecal Strepto. (c/100 mℓ)	Enteric Viruses (c/10 ℓ)	Coli-phages (c/10 mℓ)
Settled sewage (WS18)						
29 June 1982 to 21 Sept. 1982						
No. of tests	13	13	13	13	13	13
No. of positive tests	13	13	13	13	13	13
Average count	16×10^6	353×10^6	18×10^6	6×10^5	447×10^4	40×10^4
Median count	16×10^6	260×10^6	19×10^6	5×10^5	$170 \times 10_4$	40×10^4
Range of counts	$(5\text{-}27) \times 10^6$	$(125\text{-}1130) \times 10^6$	$(5\text{-}48) \times 10^6$	$(2{,}4\text{-}14) \times 10^5$	$(5{,}3\text{-}2200) \times 10^4$	$(19\text{-}69) \times 10^4$
Standard deviation	$7{,}4 \times 10^6$	261×10^6	$10{,}9 \times 10^6$	$3{,}4 \times 10^5$	766×10^4	$14{,}1 \times 10^4$
Ratio of average: phage/org.	$2{,}5 \times 10^{-3}$	0,011	0,22	6,7	89,5	
Maturation pond effluent (WR1)*						
1 Sept. 1981 to 9 Nov. 1982						
No. of tests	46	46	46	46	38	46
No. of positive tests	46	46	46	36	1	35
Average count	29×10^3	2605	42	83	0,08	32
Median count	22×10^3	235	9	1	0	2
Range of counts	$(1{,}1\text{-}86) \times 10^3$	10-38 000	0,3-240	0-1170	0-3	0-322
Standard deviation	$23{,}0 \times 10^3$	6463,7	64	227,8	0,5	71,0
Ratio of average: phage/org.	$1{,}1 \times 10^{-4}$	0,12	7,6	3,9	$4{,}0 \times 10^5$	
Goreangab Dam water (WD22)**						
9 March 1982 to 21 Sept. 1982						
No. of tests	26	26	26	26	26	26
No. of positive tests	26	26	25	26	0	7
Average count	6300	500	59	72	0	1
Median count	2600	77	6	10	0	0
Range of counts	$(2\text{-}440) \times 10^2$	1-5000	0-653	1-600	0	0-8
Standard deviation	91,7	1079,1	171,4	159,4	0	2,05
Ratio of average: phage/org.	$1{,}6 \times 10^{-5}$	0,02	0,17	0,14	>1000	
Drinking-water**						
1 Sept. 1981 to 10 May 1983						
No. of tests	45	45	45	45	45	45
No. of positive tests	45	2	0	0	1	0
Average count	603	0,2	0	0	0,02	0
Median count	63	0	0	0	0	0
Range of counts	1-5100	0-5	0	0	0-1	0
Standard deviation	1196,0	0,8	0	0	0,15	0

*Intake of reclamation plant.
**Intake of conventional drinking-water treatment plant.
***Reclaimed water (WR14), conventionally treated water (WD23), or mixture of the two.

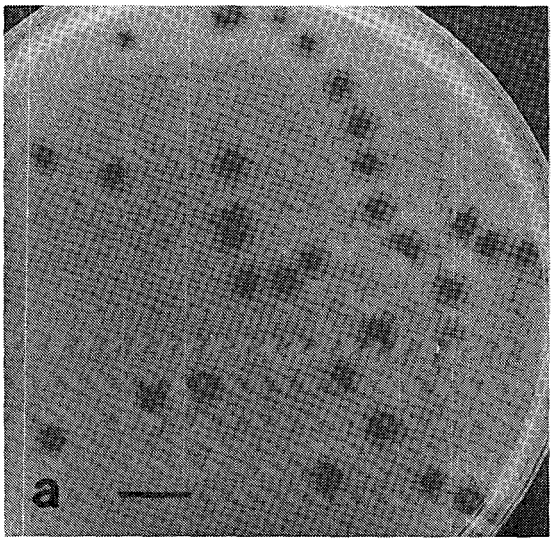
TABLE 5
COUNTS OF MICRO-ORGANISMS IN TREATED RAND WATER BOARD WATER

Determinants	Plate Count (c/1 mℓ)	Total Coliforms (c/100 mℓ)	Faecal Coliforms (c/100 mℓ)	Acid-fast Bacteria (c/100 mℓ)	Faecal Strepto. (c/100 mℓ)	Enteric Viruses (c/10 ℓ)	Coli-phages (c/10 mℓ)
Zuikerbosch Plant (B4, B6, B7, B8) 7 April 1981 to 10 May 1983							
No. of tests	64	64	64	59	64	64	64
No. of positive tests	64	0	0	37	0	0	1
Average count	19	0	0	2	0	0	0,08
Median count	7	0	0	1	0	0	0
Range of counts	1-330	0	0	0-15	0	0	0,5
Standard deviation	43,2	0	0	2,8	0	0	0,6
Ratio of average: phage/org.	$4{,}2 \times 10^{-4}$	>0,8	>0,8	0,4	> 0,8	>80	
Vereeniging Plant (A6, A8, A14) 7 April 1981 to 17 May 1983							
No. of tests	82	82	82	71	82	82	82
No. of positive tests	82	1	0	51	0	0	5
Average count	34	0,04	0	7	0	0	0,18
Median count	21	0	0	2	0	0	0
Range of counts	5-660	0-3	0	0-73	0	0	0-5
Standard deviation	72,3	0,3	0	13,3	0	0	0,8
Ratio of average: phage/org.	$5{,}3 \times 10^{-4}$	45	>1,8	0,26	>1,8	>180	

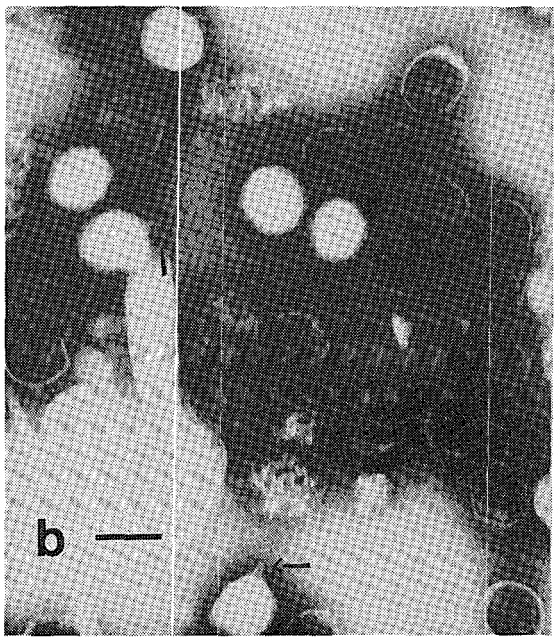
E. coli strains B or K12 *Hfr*. Coliphage V1 has a cubical head structure with a diameter of 60 nm, and a very short tail (Figure 1b).

Discussion

The differences in coliphage counts obtained in plaque assays on various waters using different host strains and incubation at 37 °C or 25 °C (Tables 1 and 2), illustrate the importance of detection techniques in the enumeration of coliphages in water environments. Although statistically of limited significance (Table 2), the coliphage assays using *E. coli* strains K12 *Hfr* and B as hosts suggest an association between phages and waters containing a high incidence of hosts of human origin (optimum growth at 37 °C) or hosts from natural environments (optimum growth at 25 °C) because settled sewage (hosts predominantly of



a. Plaques of coliphage V1 on Escherichia coli C603 after incubation for 18 h at 37 °C. Bar = 10 mm.



b. Electron micrograph of 'empty' and 'full' coliphage V1 particles in a preparation negatively stained with phosphotungstate (Bradley, 1967). Note short, non-constractile tail (arrows). Bar = 60 nm.

Figure 1

human origin) tended to yield higher counts at 37 °C while activated sludge effluent and river water (hosts predominantly from natural environments) tended to yield higher counts at 25 °C (Table 1). A similar association between phage ecology and certain water environments has been reported by Seeley and Primrose (1980). *Escherichia coli* C603 would not seem to recognize these variations in phage ecology because it tended to yield a higher count at 37 °C for all waters tested (Table 1).

The failure to detect variations in phage ecology, and the significantly higher counts generally obtained in assays using *E. coli* C603 as host compared to strains K12 *Hfr* and B, were most likely due to the absence of DNA restriction and modification systems in *E. coli* C and its derivative, strain C603 (Seeley and Primrose, 1982). Relatively high phage counts in assays using *E. coli* C603 as host were also reported by Logan *et al.* (1981). Assays using *E. coli* C603 as host and incubation at 37 °C would therefore seem to be one of the most sensitive methods presently available for counting coliphages in water. However, as a result of the complex host specificity of bacteriophages, even this system would not recover all coliphages (Seeley and Primrose, 1982). For instance, *E. coli* C603 is not susceptible to male-specific phages and group P plasmid-specific phages, which are highly prevalent in water environments (Seeley and Primrose, 1982), because it carries neither a sex factor nor the group P plasmid.

Failure to detect male-specific phages is additionally unfortunate in so far that these phages are specific for faecal pollution because they cannot infect hosts and multiply in water environments like many other phages do, as their adsorption site, the F pilus, is not formed at temperatures below 30 °C (Seeley and Primrose, 1982). In terms of total populations of coliphages detected in the waters tested in this study, male-specific phages did not seem to represent a significant portion of the numbers because counts on *E. coli* K12 *Hfr* (which carries a sex factor) were significantly lower than on *E. coli* C (Table 2), and counts on *E. coli* K12 *Hfr* were generally lower at 37 °C than that at 25 °C (Table 1). Although male-specific phages may to a large extent account for the higher numbers on *E. coli* K12 *Hfr* at 37 °C in assays on Daspoort settled sewage (Table 1), it should be noted that *E. coli* B, which does not carry a sex factor, also had higher counts at 37 °C which indicates that male-specific phages may not be a significant factor in the higher counts at 37 °C.

Information regarding coliphages recovered on *E. coli* C603 is extremely limited (Logan *et al.*, 1981; Seeley and Primrose, 1982). Coliphage V1 (Figure 1) which, at least in terms of plaque morphology, would appear to be typical of phages recovered on *E. coli* C603, does not seem to match any of the wide variety of coliphages recently described for Canadian waters (Ackermann and Nguyen, 1983), illustrating the complexity of bacteriophage ecology. Coliphage V1 appears to belong to the Group C phages of Bradley (1967) since it has a short, non-contractile tail and the head seems to have icosahedral symmetry, while the general morphology and size resembles that of coliphage T3 (Bradley, 1967). This implies that V1 probably has double-stranded DNA nucleic acid (Bradley, 1967).

Although the correlation between counts of coliphages and coliform bacteria reported by Wentsel *et al.* (1982) and Zaiss (1982) is confirmed by results for some of the waters tested, the data on average counts, median counts, standard deviations and ratios of coliphages to coliforms and other organisms (Tables 3 to 5) show that such correlations may not be meaningful for all waters. Differences in the survival and multiplication of phages and bacteria in various water environments are considered an important reason for fluctuating ratios of coliphage to coliform counts reported in several studies (Table 3 to 5; Bell, 1976; Berg, 1978). Likewise, differences can be expected in the ratios of counts of coliphages and enteric viruses in different water environments, and have been illustrated in practice (Tables 3 to 5; Kott *et al.*, 1974; Vaughn and Metcalf, 1975; Berg, 1978). Ratios between various bacteria, coliphages and enteric viruses are particularly subject to fluctuations in the case of raw, treated and disinfected water supplies as a result of considerable differences in the resistance of different micro-organisms to physico-chemical and disinfection processes (Berg, 1978; Grabow *et al.*, 1980, 1983; IAWPRC Study Group on Water Virology, 1983).

Although coliphage counts may not always serve as a reliable indicator of numbers of enteric viruses and other organisms in various water environments as a result of fluctuating ratios, they have the following three additional indicator features:

1. For all practical purposes coliphages consistently outnumbered enteric viruses in all waters tested by a factor of at least 1 000 (Tables 3 to 5). The higher incidence of coliphages is substantiated by the fact that 211 samples yielded positive results for coliphages and negative results for enteric viruses, while only two samples were positive for enteric viruses and negative for coliphages, even though coliphage tests were always done on 10 mℓ samples while enteric virus tests were done on 10 ℓ samples, using the most efficient virus recovery and detection methods presently available (Nupen *et al.*, 1980; Grabow and Nupen, 1981; Grabow *et al.*, 1982b). Although the recovery of an enteric virus, but no coliphages, from a sample of Windhoek drinking-water (Table 4) cannot be disregarded, indications are that this isolate was a contaminant. Among the reasons are that enteric viruses were rarely detected in the raw waters used for the preparation of the drinking-water (Table 4), the treatment processes concerned can inactivate enteric viruses efficiently (Grabow, 1977; Grabow *et al.*, 1978, 1980, 1983), all indicator tests confirmed satisfactory water treatment and plant operation, and the isolate was an untypable enterovirus which implies that it is not a virus commonly encountered in water. In view of the general quality of the water concerned, the sample of A9 Vaal River Barrage Water (Table 3) which yielded an enteric virus but no coliphages, was possibly also contaminated, or an example of a rare result due to extreme fluctuations in numbers of different organisms, or the release of adsorbed enteric viruses from bottom sediments. Contamination of samples is a factor which has to be considered in microbiological water quality evaluation, particularly when sampling and handling large volumes of water as in the case of viral tests (Hoehn and Randall, 1981), and is one reason why water quality standards generally specify that a certain percentage of samples should meet a certain limit (SABS, 1971). The generally much higher incidence of coliphages than enteric viruses in water environments is substantiated by many other studies (Kott *et al.*, 1978; Berg, 1978; Grabow *et al.*, 1980; IAWPRC Study Group on Water Virology, 1983).
2. Coliphages are generally more resistant than enteric viruses to unfavourable environmental conditions as well as water treatment and disinfection processes. This has been established by extensive field and laboratory studies (Kott *et al.*, 1974, 1978; Berg, 1978; Grabow, 1977; Grabow *et al.*, 1978, 1980, 1983; IAWPRC Study Group on Water Virology, 1983) and is illustrated by the increased ratio of coliphages to enteric viruses in Windhoek settled sewage after maturation pond treatment (Table 4), and possibly also by the occasional detection of coliphages in treated Rand

Water Board water (Table 5). The incidence of coliphages in treated drinking-water supplies was generally exceeded only by that of plate count and acid-fast organisms (Tables 4 and 5), which shows that these organisms were even more resistant to the disinfection processes concerned. Under certain circumstances the ratio of coliphages to enteric viruses may be affected by the multiplication of coliphages in suitable water environments, which to a certain extent increases the indicator value of coliphages (Grabow *et al.*, 1980; Seeley and Primrose, 1982).

3. Coliphages can be counted by simple and inexpensive methods which do not require the processing of large volumes of water and yield results within 6 to 18 h while enteric virus tests may take up to three weeks (Kott *et al.*, 1978; Berg, 1978; Grabow *et al.*, 1978, 1980; Wentsel *et al.*, 1982; IAWPRC Study Group on Water Virology, 1983).

The above three features of coliphages imply that they meet the basic requirements of indicators for evaluating the virological safety of water (Berg, 1978), and that their absence from treated water supplies offers virtually conclusive evidence of the absence of enteric viruses. The safety margin of coliphage tests can be increased even further by improving the sensitivity of recovery and detection methods, and research along these lines is in progress. The findings of this and earlier work (IAWPRC Study Group on Water Virology, 1983) indicate that coliphage assays in combination with the highly responsive standard plate count, coliform tests for the detection of faecal pollution, and counts of the highly resistant acid-fast bacteria, eliminate the need for virological analyses on treated drinking-water supplies, even in the case of the direct reclamation of drinking-water from wastewater.

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