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COLIPHAGE COUNTS, ARE THEY NECESSARY TO MAINTAIN DRINKING WATER SAFETY

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ABSTRACT

Samples of drinking water collected from three different sources were tested for total coliforms, fecal coliforms and coliphage concentrations. Five samples from each site were tested with and without dechlorination procedures. Results showed that nine of the samples were positive for coliphage while being negative for total and fecal coliforms, another nine samples were positive for all three parameters. In no instance were total coliform and fecal coliform counts found without coliphage also being found.

Results of this study indicate that coliform counts are not fully protective of treated drinking water and the presence of coliphage in these waters could be an indication of the presence of other viruses.

RÉSUMÉ

On a déterminé les concentrations de coliformes totaux, de coliformes fécaux et de coliphages dans des échantillons d'eau potable prélevés à trois endroits différents. Cinq échantillons de chacun des sites ont été étudiés avec et sans utilisation de la méthode de déchloration. Les résultats montrent que neuf des échantillons renfermaient des coliphages mais ne renfermaient pas de coliformes totaux et fécaux, tandis que neuf autres échantillons affichaient des résultats positifs pour ces trois paramètres. En aucun cas, on n'a relevé de coliformes totaux et de coliformes fécaux sans trouver en même temps des coliphages.

Les résultats de cette étude montrent que les dénombrements de coliformes ne sont pas suffisants pour assurer la qualité de l'eau potable traitée et que la présence de coliphages dans ces eaux pourrait indiquer la présence d'autres virus.

MANAGEMENT PERSPECTIVE

Coliphages are viruses which infect E. coli and other fecal coliform bacteria. Microbiology Laboratories Section, NWRI has been evaluating and using coliphages as surrogate indicators of fecal pollution in receiving waters because the test is simple, inexpensive and, most importantly, the samples can be preserved for at least 72 hours before testing. Thus opening up a greater portion of Canadian waters for biomonitoring.

Since coliphages are viruses, their reactions to disinfection whether in the sewage treatment plant or drinking water treatment plant, are similar to other viruses. Thus the finding of coliphages in drinking water also implies that human pathogenic viruses can also be present as the disinfection treatment was inadequate to remove coliphages.

The data for this report were obtained from an IDRC (International Development Research Centre, Ottawa) funded study in South East Asia, for which the co-author, B.J. Dutka was the study originator and consultant.

PERSPECTIVE-GESTION

Les coliphages sont des virus qui infectent *E. coli* et autres bactéries coliformes fécales. La Section de laboratoires de microbiologie de l'Institut national de recherche sur les eaux évalue l'utilité des coliphages comme indicateurs substituts de la pollution fécale dans les eaux réceptrices parce que ce test est simple, peu coûteux et, surtout que les échantillons peuvent être conservés au moins 72 heures avant d'être soumis à des analyses. Ainsi, une plus grande portion des eaux canadiennes pourrait faire l'objet de bio-surveillance.

Étant donné que les coliphages sont des virus, leur réaction à la désinfection, dans la station de traitement des eaux usées ou dans la station de traitement de l'eau potable est semblable à celle d'autres virus. S'il y a présence de coliphages dans l'eau potable, cela veut dire qu'il pourrait également y avoir d'autres virus pathogènes pour l'humain puisque le traitement de désinfection n'a pas éliminé les coliphages.

Les données présentées ici ont été obtenues dans le cadre d'une étude réalisée dans le sud-est asiatique et subventionnée par le Centre de recherche pour le développement international à Ottawa.
D.J. Dutka, co-auteur du présent rapport, a été concepteur de cette étude et consultant.

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INTRODUCTION

The health and well-being of the majority of the worlds' population is dependent to a large extent on the quality of water they drink. In order to prevent rapid spread of waterborne diseases, community water samples must be treated to remove bacterial, viral and parasitic contamination that causes these diseases.

The safety of drinking water has been traditionally controlled by chlorination and coliform count estimation. The accepted practice is to test drinking water after treatment, including disinfection, at various points in the distribution system and if the water is found to be coliform free, the water is deemed safe for consumption. This practice has apparently worked universally to provide safe pathogen-free drinking water.

However, during a recent study on the distribution patterns of coliphage in raw and treated drinking water, data were obtained which tend to challenge the accepted view that a coliform-free drinking water is a safe pathogen-free drinking water. This report describes the study and data obtained.

METHODS

Samples

Water samples were collected from three different taps, over a two month period, within the campus grounds of the National University of Singapore. Fifty percent of the samples were dechlorinated with the addition of appropriate amounts of 10% sodium thiosulfate (APHA 1986). The other 50 percent were tested without dechlorination procedures. All samples were processed within three hours of collection. Although Singapore routinely chlorinates all potable waters before distribution, residual chlorine levels were not obtained on the waters tested.

Microbiological Procedures

The water samples were tested for total coliforms, fecal coliforms and coliphage concentrations. Total coliform density estimations were obtained by membrane filtration procedures using m Endo agar (37°C) and by the five tube MPN procedures using Lauryl Sulfate Tryptose (LST) broth with confirmation in 2% Brilliant Green Bile Broth (BGB) (37°C) (APHA 1986). Fecal coliform populations were estimated by two MPN (5 tube) procedures, Al broth (24 hr at 44.5°C) and the LST, BGB procedure with confirmation in EC broth (24 hr at 44.5°C) (APHA 1986).

Coliphage are bacterial viruses (bacterophage) which infect and replicate in lactose fermenting Enterobacteriaceae (coliform and fecal coliforms). Since coliphages replicate only in coliform and fecal coliform organisms, the presence of coliphage in waters also indicates the probable presence of these indicators. The procedure used in these studies to estimate coliphage concentrations is that found in Section 919C of 16th Edition APHA Standard Methods (1986).

Six and twenty-four hour coliphage counts were performed as detailed (APHA 1986). The *E. coli* host used in these studies was *E. coli* c, the strain selected by the Atlantic Research Corporation (Scott *et al*. 1979). The plaque reaction was enhanced by the addition of 2,3,5-triphenyltetrazolium chloride to the medium.

RESULTS AND DISCUSSION

All data obtained during this study are tabulated in Table 1. Here it can be seen that, with two exceptions, (6 hr - ML2 and M15) all potable water samples tested were positive for coliphage after 24 hours incubation. No samples, chlorinated or dechlorinated, were positive for total coliforms or fecal coliforms and negative for coliphage.

In examining the efficiency of MF versus MPN techniques to resucitate and grow total coliforms from tap water, it can be seen that in normal chlorinated samples the MF and MPN techniques were each positive three times when the other was negative. In the remainder of the samples, the MF and MPN gave similar responses.

In the dechlorinated samples, four MF and one MPN procedure were positive while the other was negative, whereas in the remainder of the samples they gave similar responses. The implications of these few results are that, at best, the MF technique may be more sensitive in enumerating total coliforms from drinking waters, realizing that the MF technique screens 100 mL of water and the MPN technique only 55.5 mL (5 x 10 mL, 5 x 1 mL, 5 x 1 mL). In practice it would appear that either technique could be equally successful in estimating coliform presence and total coliform populations.

In evaluating the potential of coliphage to be used as an indicator of possible hazards in drinking waters, it can be seen that in the 30 samples tested, nine were positive for coliphage and negative for total and fecal coliforms.

Studies by Scott *et al.* (1979) have shown that coliform bacteria (*E. coli*) survived a 6 mg/L dose of chlorine for five minutes while coliphages survived in large numbers even after exposure to 25 mg/L chlorine for 80 minutes. Thus, while chlorine disinfection effectively kills coliform bacteria, coliphages survive in large numbers (Scott *et al.* 1979).

In an extensive review by Grabow (1968) data are reviewed and presented which strongly suggest that most common pathogenic viruses are much more resistant to chlorination treatment than are *E. coli*. These reports were also confirmed by Chambers (1971) and Havelaar (1986) in studies on sewage treatment plant effluents. Thus the findings of coliphage in these drinking water samples, with and without coliform and/or fecal coliform presence, is suggestive that pathogenic viruses can also survive the normal treatment and disinfection process accorded these potable water samples (Havelaar 1986).

Another implication of the data from these studies is that coliform-free potable waters are not necessarily pathogen-free potable waters.

Based on these limited studies, the authors suggest that coliphage tests be included as part of any potable water testing scheme.

The coliphage test has an advantage over traditional microbiological tests, in that the test can be read after six hours of incubation, if necessary. It is very economical and simple to perform and its sensitivity can easily be increased by testing more 5 mL aliquots or by using a coliphage MPN technique.

REFERENCES

APHA. 1986. Standard methods for the examination of water and wastewater. 16th Ed. American Public Health Associates, Washington, D.C.

Chambers, C.C. 1971. Chlorination for control of bacteria and viruses in treatment plant effluents. Journal Wat. Poll. Cont. Fed. 43(2):228-241.

Grabow, W.O.K. 1968. Review Paper: The virology of wastewater treatment. Water Research. 2:675-716.

Havelaar, A.H. 1986. F-specific RNA bacteriophages as model viruses in water treatment processes. Published by Rijkinstituut voor Volksgezonheid en Milieuhygiëne, Bilthoven, Netherlands, 240 pp.

Scott, W.M., O'Neill, P.E., Wilkinson, M.J. and Kitchens, J.F. 1979. Evaluation of coliform bacteria and bactereophage relationships in assessment of water quality. Technical Report, NSF Grant No. PFR28-19196, Atlantic Research Corporation, Alexandria, Virginia.

Table 1 **Incidence of Coliforms, Fecal Coliforms and Coliphage in Chlorinated and Dechlorinated Drinking Water**

Chlorinated Water

Source	Total Coliform/100 mL		Fecal Coliform/100 mL			Total Coliphage/100 mL	
	MF Endo	MPN LST	mFC	E.C.	A1	6 hr	24 hr
M1[1]	0	4	0	2	<2	<5	50
M2	0	<2	0	<2	<2	<5	275
M3	5	<2	0	<2	<2	<5	50
M4	0	<2	0	<2	<2	<5	100
M5	1	<2	0	<2	<2	<5	50
L1[2]	0	<2	0	<2	<2	<5	<5
L2	3	2	0	<2	<2	<5	425
L3	1	4	0	2	4	<5	125
L4	1	<2	0	<2	<2	<5	200
L5	0	4	0	4	<2	<5	5
ML1[1]	1	4	0	2	<2	<5	5
ML2	0	<2	0	<2	<2	25	575
ML3	9	6	9	2	2	<5	40
ML4	0	<2	0	<2	<2	<5	50
ML5	0	4	0	2	<2	<5	25

Tap Water Dechlorinated with 10% Sodium Thiosulphate

Source	MF Endo	MPN LST	mFC	E.C.	A1	6 hr	24 hr
M11	3	<2	0	<2	<2	<5	50
M12	0	<2	0	<2	<2	<5	175
M13	7	<2	0	<2	<2	<5	100
M14	0	<2	0	<2	<2	<5	75
M15	1	<2	0	<2	<2	25	25
L11	4	<2	0	<2	<2	<5	50
L12	0	2	0	2	<2	<5	25
L13	7	4	6	2	4	<5	300
L14	0	<2	0	<2	<2	<5	75
L15	0	<2	0	<2	<2	<5	50
ML11	0	<2	0	<2	<2	<5	0
M112	2	2	0	<2	<2	<5	125
ML13	15	2	14	2	2	<5	50
ML14	0	<2	0	<2	<2	<5	275
ML15	0	<2	0	2	<2	<5	25

[1] M = micro industrial water tap
[2] L = library water tap
[3] ML= medical water tap