

INCIDENCE OF COLIPHAGE IN  
POTABLE WATER SUPPLIES

by

M.M. El-Abagy<sup>1</sup>, B.J. Dutka<sup>2\*</sup>,  
and M. Kamel<sup>1</sup>

<sup>1</sup>Water Pollution Control Laboratory  
National Research Centre  
Dokki, Cairo, Egypt

<sup>2</sup>Rivers Research Branch  
National Water Research Institute  
Canada Centre for Inland Waters  
Burlington, Ontario, L7R 4A6

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\*Author to whom all correspondence should be sent.

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<sup>1</sup>Water Pollution Control Laboratory  
National Research Centre  
Dokki, Cairo, Egypt

<sup>2</sup>Ecotoxicology and Biomonitoring Project Team  
Rivers Research Branch  
National Water Research Institute  
Canada Centre for Inland Waters  
P.O. Box 5050  
Burlington, Ontario, Canada L7R 4A6

\*Author to whom all correspondence should be sent.

## ABSTRACT

Samples of drinking water, from different sources in greater Cairo, Egypt, and bottled drinking water were tested for total coliform, fecal coliform and coliphage populations. Of the 147 samples tested, four samples were positive for both total coliforms and coliphage, 65 samples were negative for total coliforms, fecal coliforms and coliphage and 78 samples were positive for coliphage and negative for total coliforms and fecal coliforms. The incidence of coliphage in these potable water supplies reflects the probability of human pathogenic virus presence.

## RÉSUMÉ

Des échantillons d'eau potable prélevés en différents endroits de la région métropolitaine du Caire en Egypte et des échantillons d'eau de boisson embouteillée ont fait l'objet d'essais pour doser les coliformes totaux, les coliformes fécaux et les populations de coliphages. Sur les 147 échantillons étudiés, quatre ont donné des résultats positifs pour les coliformes totaux et les coliphages, 65 des résultats négatifs pour les coliformes totaux, les coliformes fécaux et les coliphages, et 78 des résultats positifs pour les coliphages et négatifs pour les coliformes totaux et les coliformes fécaux. L'incidence des coliphages dans ces approvisionnements en eau potable témoigne de la présence probable de virus pathogènes pour les humains dans ces eaux.

## MANAGEMENT PERSPECTIVE

Coliphages are viruses which infect E. coli and other fecal coliform bacteria. Ecotoxicology and Biomonitoring Project Team, 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 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 Egypt, for which the co-author, B.J. Dutka was the study originator, consultant and report writer.

## PERSPECTIVES DE GESTION

Les coliphages sont des virus qui infectent E. coli et d'autres bactéries coliformes fécales. Le groupe de recherche en écotoxicologie et en surveillance biologique de l'INRE évalue les coliphages depuis un certain temps et s'en sert comme indicateurs substitués de pollution fécale dans les eaux réceptrices parce que leur dosage est simple, peu coûteux et, ce qui est plus important, parce qu'on peut garder les échantillons pendant au moins 72 heures avant de les soumettre aux essais, ce qui permet d'exercer une surveillance biologique sur une plus grande proportion des eaux canadiennes.

Comme les coliphages sont des virus, leurs réactions à la désinfection, que ce soit à l'usine de traitement des eaux usées ou à l'usine d'épuration, sont semblables à celles des autres virus. Par conséquent, la présence de coliphages dans les eaux potables implique que des virus pathogènes pour les humains peuvent se trouver dans l'eau, étant donné que le traitement de désinfection n'a pas éliminé les coliphages.

Les données contenues dans ce rapport ont été obtenues au moyen d'une étude financée par le CRDI (Centre de recherches sur le développement international, Ottawa) en Egypte, pour laquelle le co-auteur, B.J. Dutka, agissait à titre de promoteur, de consultant et de rédacteur.

## INTRODUCTION

The safety of drinking water is an ongoing concern in all countries. Traditionally the safety of potable water supplies has been controlled by disinfection, usually by chlorination, and by coliform population estimates. However, in a recent study (9) it was shown that a coliform free potable water may not necessarily be free of microbial pathogens. The data from this study (9) indicated that, while many of the treated potable waters sampled may have been free of coliforms, they did contain varying concentrations of coliphage, an indication of inadequate water treatment and a warning that human enteric viruses may also be present.

Studies by the Atlantic Research Corporation scientists (7) on the use of coliphage as an indicator of fecal pollution has greatly advanced the knowledge on the usefulness of this water quality indicator. There is also sufficient evidence to suggest that the coliphage test has many advantages over traditional bacteriological and virological tests, in that the procedure is economical, simple to perform and provides results within six hours.

In this report, we present the results of an investigation into the safety of a variety of Cairo, Egypt, drinking water samples collected from distribution lines, storage tanks, wells and bottled waters. The indicator systems used to test for the safety of these waters were total coliform, fecal coliform and coliphage population estimates.

## MATERIALS AND METHODS

### Samples

Triplicate drinking water samples were collected from a variety of sources within Greater Cairo, Egypt, usually monthly over a ten-month period (October 1986 - July 1987). The samples were collected from three water treatment plants (Tebbin, Giza and Rod El-Farag), four deep wells located at Mastorod and six storage tanks from various locations which are used to provide drinking water to high rise buildings. Three brands of bottled water, with the following trade names were also tested; Baraka, Helwan and Mineral. Water samples collected from the water treatment plants and storage tanks were dechlorinated with thiosulphate (0.1 g/litre of sample). All samples were processed within two hours of collection.

### Microbiological Procedures

Two coliform MPN and two fecal coliform MPN procedures were used on all water samples. In one of the coliform procedures, a 10 tube MPN procedure using lauryl tryptose sulfate broth (35°C for 48 hr) (1) with confirmation in brilliant green lactose bile broth (35° for 48 hrs) was used and in the other 10 tube MPN procedure MacConkey broth (35°C for 48 hr) was confirmed by subculturing positive tubes to Levine EMB agar. Fecal coliform populations were estimated by the 10 tube MPN procedure using A1 broth (44.5°C for 24 hr) (1), the 10 tube MPN series using lauryl tryptose sulfate broth (35°C for 48 hr) followed by brilliant



green lactose bile broth (35°C for 48 hr) with positive tubes being confirmed in EC broth (44.5°C for 24 hr) (1).

Two procedures were used to estimate coliphage concentrations. One procedure is that described in section 919C, Standard Methods (1) with the addition of 2,3,5-triphenyl tetrazolium chloride and using E. coli C (ATCC #13706) as host. The other procedure used was the MPN technique as recommended by Kott (4) using E. coli C as host.

#### RESULTS AND DISCUSSION

Selected representative results are presented in Table 1. Here the mean values of triplicate tests are presented for those samples which indicated that coliforms were not present in the potable waters tested. Thus, out of the total 147 samples tested, 78 samples were positive for coliphage and negative for total coliforms and fecal coliforms, 65 samples were negative for total coliforms, fecal coliforms and coliphage and 4 samples were positive for both total coliforms and coliphage. Two of these 4 positives were found in the bottled water labelled "Mineral" and both fecal and total coliforms were recovered. In the other two samples, well 20 and well 31, no fecal coliforms were recovered.

Several researchers (6, 7) have demonstrated that coliphages are more resistant to inactivation by chlorination than coliforms. In one study (7) it has been shown that E. coli were non viable after a 5 minute contact with 6 mg chlorine/L while coliphages were able to survive an exposure of 25 mg chlorine/L for 80 minutes. Thus, while chlorine effectively kills coliform bacteria, coliphages survive in large numbers (7).

In an extensive review of the early literature, Grabow (2) found that the results of many studies indicated that most common pathogenic viruses are more resistant to chlorination than are E. coli. Then, in a later study, Kott<sup>5</sup> reported that coliphage are similarly resistant to chlorination as polioviruses. Thus the finding of coliphage in these drinking water samples with and without coliform presence, strongly suggests that viruses can also survive the normal treatment and disinfection process accorded these potable water samples (3). Another implication of the data from these studies is that coliform-free potable waters are not necessarily pathogen-free potable waters.

The findings reported here re coliform free but coliphage containing potable waters are not single rare events. Similar results have also been reported from Singapore potable water supplies (9).

Another interesting finding in this study is the great superiority of the APHA coliphage procedure over the Kott (4) MPN procedure even though both techniques used the same E. coli C host. Moreover it was also found there was closer agreement between the triplicate APHA coliphage results than the Kott (4) procedure coliphage results. From these data and other data using the APHA coliphage technique (1), it would appear this method is at present the best available technique for coliphage estimation and it has the added benefit of being a relatively simple inexpensive procedure which can produce results within 6 hours.

In summary, we believe, based on these and earlier reported data (9) that the coliphage test should be included as part of any potable water testing scheme to protect the unwary consumer.

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REFERENCES

1. APHA. 1985. Standard methods for the examination of water and wastewater. 16th Ed. American Public Health Associates, Washington, D.C.
2. Grabow, W.O.K. 1968. Review Paper: The virology of wastewater treatment. Water Research. 2:675-716.
3. Havelaar, A.H. 1986. F-specific RNA bacteriophages as model viruses in water treatment processes. 240 pp, Bilthoven, Netherlands: Rijkinstituut voor Volksgezondheid en Milieuhygiëne.
4. Kott, Y. 1966. Estimation of low numbers of Escherichia coli bacteriophage by use of the most probable number method. Appl. Microbiol. 14: 141-145.
5. Kott, Y., N. Roze, S. Sperber and N. Betzer. 1974. Bacteriophage as viral pollution indicators. Wat. Res. 8: 165-171.
6. Longley K.K., B.E. Moore, and C.A. Sorber. 1980. Comparison of chlorine and chlorine dioxide as disinfectants. J. Wat. Poll. Cont. Fed. 52: 2098-2105.

7. Scott, W.M., P.E. O'Neill, M.J. Wilkinson and J.F. Kitchens. 1979. Evaluation of coliform bacteria and bacteriophage relationships in assessment of water quality. Technical Report, NSF Grant No. PFR28-19196, Atlantic Research Corporation, Alexandria, Virginia.
8. Seeley, N.D. and S.B. Primrose. 1982. The isolation of bacteriophages from the environment. J. Appl. Bact. 53: 1-17.
9. Sim, T.S. and B.J. Dutka. 1987. Coliphage counts: are they necessary to maintain drinking water safety. MIRCEN J. (in press).

Table 1 Incidence of coliphage in drinking water containing no coliforms based on triplicate samples

Source	Coliphage/100 mL				MPN-index/100 mL			
	APHA (1)		Kott (4)		Total Coliform		Fecal Coliform	
	No. of Positives	Range of Means	No. of Positives	Range of Means	LST	EMB	EC	AI
Tebbin <sup>1</sup>	4/10	10-330	1/10	2	0	0	0	0
Giza <sup>1</sup>	7/10	3-600	0/10	0	0	0	0	0
Rod-El Faraq <sup>1</sup>	5/10	4-800	0/10	0	0	0	0	0
Storage Tank 1 <sup>1</sup>	4/10	13-94	1/10	2	0	0	0	0
Storage Tank 2 <sup>1</sup>	4/10	7-150	0/10	0	0	0	0	0
Storage Tank 3 <sup>1</sup>	5/10	3-700	0/10	0	0	0	0	0
Storage Tank 4 <sup>1</sup>	3/10	10-870	1/10	1	0	0	0	0
Storage Tank 5 <sup>1</sup>	1/10	90	0/10	0	0	0	0	0
Storage Tank 6 <sup>1</sup>	1/8	7	0/8	0	0	0	0	0
Well 20 <sup>2</sup>	7/8	3-33	6/8	2-7	0	0	0	0
Well 21 <sup>2</sup>	7/9	10-770	4/9	3-12	0	0	0	0
Well 31 <sup>2</sup>	10/10	13-2200	5/10	3-20	0	0	0	0
Well 32 <sup>2</sup>	7/10	7-1140	3/10	3-7	0	0	0	0
Baraka <sup>2</sup>	5/7	3-47	1/7	3	0	0	0	0
Helwen <sup>2</sup>	3/7	3-10	0/7	0	0	0	0	0
Mineral <sup>2</sup>	5/8	27-100	3/8	5-8	0	0	0	0
Total	78/147		25/147		0/147	0/147	0/147	0/147

<sup>1</sup>Chlorinated water samples dechlorinated before investigation.

<sup>2</sup>Samples not chlorinated.