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COLIPHAGE PRESENCE IN VARIOUS TYPES OF POTABLE WATER IN THAILAND

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ABSTRACT

Three types of drinking water used in Thailand, tap water, rain water and deep well water (200m) were tested for bacteriological and coliphage content. 13.5% of the samples contained only coliphage and 13% contained both coliphage and total coliforms. The incidence of coliphage in these potable water supplies reflect the probability of human pathogenic virus also surviving in these waters. The H_2S paper-strip test was found to be an equally sensitive indicator of coliform presence compared to MF and MPN total coliform procedures.

RÉSUMÉ

On a mesuré la quantité de bactéries et de coli-phages dans trois sortes d'eau potable utilisées en Thaïlande, l'eau du robinet, l'eau de pluie et l'eau provenant de puits profonds (200 m). Treize et demi pour cent des échantillons contenaient des coli-phages seulement et 13 % contenaient à la fois des coli-phages et des coliformes totaux. Le taux de coli-phages dans ces réserves d'eau potable indique la probabilité que des virus pathogènes humains survivent aussi dans ces eaux. On a trouvé que l'analyse au papier réactif H_2S était un indicateur de la présence de coliformes aussi sensible que l'analyse par filtre à membranes et le M.P.N.

MANAGEMENT PERSPECTIVE

In developing countries and isolated areas in developed countries, the deliverance of potable water which is safe, adequate and accessible to all is not an easy task and rarely accomplished. Limited resources must be directed toward achieving an optimum balance between these needs. An important consideration in the development and maintenance of safe water supplies is the use of appropriate and simple monitoring technology for assessing microbiological water quality.

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 Thailand, for which the co-author, B.J. Dutka was the study proposer consultant and report writer.

PERSPECTIVE-GESTION

Dans les pays en voie de développement et dans les régions isolées des pays développés, la distribution en quantité suffisante d'une eau potable sûre et accessible à tous est une tâche difficile qui est rarement accomplie. Il faut utiliser les ressources limitées de façon à atteindre l'équilibre optimal entre ces trois conditions. L'utilisation d'une technologie de contrôle simple et appropriée permettant d'évaluer la qualité microbiologique de l'eau est un aspect important de la mise en place et de la conservation de réserves d'eau sûre.

Les coli-phages sont des virus qui infectent E. coli ainsi que d'autres bactéries coliformes fécales. L'équipe du projet d'écotoxicologie et de surveillance biologique de l'INRE a évalué l'utilisation des coli-phages comme substituts pour mesurer le degré de pollution fécale des eaux réceptrices parce que l'analyse est simple, peu coûteuse et, surtout, les échantillons peuvent être conservés pendant au moins 72 heures avant d'être analysés, ouvrant ainsi une plus grande partie des eaux canadiennes à la surveillance biologique.

Les coli-phages étant des virus, leurs réactions à la désinfection, que ce soit dans une usine de traitement des eaux usées ou dans une usine de traitement de l'eau potable, sont semblables à celles d'autres virus. Ainsi, la découverte de coli-phages dans l'eau potable implique que des virus pathogènes humains peuvent aussi s'y trouver étant donné que le traitement de désinfection n'a pas suffi à éliminer les coli-phages.

Les données du présent rapport proviennent d'une étude subventionnée par le CRDI (Centre de recherches pour le développement international, Ottawa) et effectuée en Thaïlande. Le co-auteur, B.J. Dutka, était l'expert-conseil de l'étude et c'est lui qui a rédigé le rapport.

INTRODUCTION

In developing countries the deliverance of potable water which is safe, adequate and accessible to all is not an easy task and rarely accomplished. Limited resources must be directed toward achieving an optimum balance between these needs. An important consideration in the development and maintenance of safe water supplies is the use of appropriate monitoring technology for assessing microbiological water quality.

It is generally acknowledged that due to increasing stresses on water supplies and rising analytical costs, the need exists to develop cheaper, simpler and quicker indicator systems which will reflect both bacterial and viral contamination of potable and raw waters.

To this end, the Department of Sanitary Engineering Mahidol University, participated in the three continent, eight country study, sponsored by The International Development Research Centre (IDRC), Ottawa, Canada, to investigate the potential use of coliphage counts to monitor the quality of raw and potable waters.

Another goal of the IDRC study was to select one or more microbiological tests which were simple, reliable and could be carried out by minimally trained personnel under minimal laboratory conditions.

In this report we present the results of an investigation into the safety of three types of drinking water currently used in Thailand, tap, rainwater and deep well water and the ability of two simple water procedures, coliphage test (APHA 1985) and the H_2S paper strip (Manja et al., 1982) to protect consumers.

METHODS

Water Samples

Thailand is in the monsoon belt and therefore has a long rainy season, usually from May to October. During the rainy season the Thai people collect rainwater from the roofs and store the water in large cisterns. These containers, (103) were sampled as one of the drinking water sources. Another source of drinking water for the rural populations were deep drilled wells, (approximately 200 metres) with casings made from galvanized steel or PVC and are either electrically or hand pumped, and a total of 37 of these potable water sources from the north eastern part of Thailand were tested (Sivaborvorn 1988). The final source was the piped water supply at Mahidol University and other buildings in Bangkok. At all sites the water was collected in 1 litre sterilized glass bottles, put into an ice-box for transport and processed within 6 hours of collection.

Microbiological Tests

All water samples were subjected to the APHA Standard Methods (1985) five tube most-probable number (MPN) technique and membrane filtration procedure (MF). These waters were also tested for coliphage using the procedure described by Wentsel et al., (1982) with the addition of 2,3,5 triphenyl tetrazolium chloride and using E. coli C (ATCC No. 13706) as host. On 21 deep well water samples the H_2S paper strip technique (Manja et al., 1982) using chemically inoculated paper strips incubated at room temperature for up to 5 days was also used to test the water supply for contaminating bacteria.

Chemical Tests

Free residual chlorine was assessed in all samples collected frin distribution lines using the amperometric titration procedures (APHA 1985)

Results and Discussion

No free residual chlorine was found in any of the distribution line samples. It is known that the water distributed through the Bangkok piped system is chlorinated at the source. However the old distribution pipes ≈ 40-50 years, leak and are heavily encrusted, and thus the chlorine is soon used up.

The coliform and coliphage data from the three potable water sources are summarized in Table 1. Here it can be seen that of the collected rain water samples 14.5% contained coliphage, 89.3% contained total coliforms and only 9.7% were coliform and coliphage free. Since the roofs of the houses are contaminated with wind blown dust and bird and animal droppings it is not surprising that only 10 samples (9.7%) were totally free of indicator organisms. And since the probability of this water being contaminated with human feces is small, perhaps the low coliphage incidence in these samples is related to the specificity of the coliphage (if present) to rodent and avian coliforms.

In the deep wells samples only 12 or 32% of the samples were negative for coliforms and coliphage while 40.5% of the samples were positive for coliphage and coliforms. Coliphage and coliforms were each the sole contaminating organisms in 10 different wells, with only 5 wells having both indicators. In this context the presence of coliphage indicate that a unsuspected hazard may exist in these coliform free potable waters.

Water drawn from various taps in Bangkok was found to be free of both coliforms and coliphage is only 37.5% of the samples while 42.5% (31) of the samples contained total coliforms and 31.2% (25) of the samples contained coliphage.

Twenty percent (16) of the samples contained only coliphage. The presence of coliphage in these drinking water samples strongly suggests that viruses can also survive in these potable waters even when normal treatment and disinfection, has taken place.

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

Studies by Petrovicova et al., 1988 have produced similar results in Czechoslovakia waters and they state "Presence of coliphages in drinking waters is an indication for aimed microbiological examination". Furthermore, since 1987, the coliphage enumeration procedure as a means of water quality control has been adopted by the Hygiene Services and Water Supply Authorities in Slovakia. Also Simkova and Cervenka (1981) have reported that "observations on coliphages appear to give a good indication of polio viruses survival in a given water system; but further verification is needed to confirm this relationship in respect to other entero viruses." Therefore on a speculative note, based on our finding 27 water samples with coliphage as the only indicator system organism presents, perhaps the presence of coliphage in water systems are indicators of the mode of transport of many human viral infections requiring the oral route for infections to occur.

In Table 2, the results of 21 deep well water samples tested by total coliform (MF and MPN) procedures, coliphage and H_2S paper strip tests are shown. In these samples, 6 contained only coliphage, 3 were only positive by the H_2S paper strip technique and only 1 sample D9 was positive for total coliforms only and that was by the MPN procedure. In this series of samples, 10 were postive by the H_2S paper strip technique indicating the presence of Enterobacteriaceae and 11 samples were positive for coliphage.

The H_2S paper strip technique data are interesting as they indicate that the procedure is equally or more sensitive for pollution indicator bacteria, as are traditional total coliform MPN and MF procedures.

Similar results were found by (Ratto et al.,(1989) and El-Abagy et al., (1988). The H_2S paper strip test is extremely simple to carry out by even minimally trained staff. This procedure is also much more cost effective that traditional TC MPN and MF tests.

In summation, the findings reported here of coliform-free but coliphage-containing potable waters are not single rare events. Similar results have been reported in studies of Singapore (Sim & Dutka 1987), Peru (Ratto et al., 1989), Egypt (El-Abagy et al., 1988). Czeckoslovakia (Petrovicova et al., 1988) and Canada (Palmateer et al., 1989 and Dutka et al., 1989), potable water supplies. Based on these and earlier studies (Sim & Dutka 1987, El-Abagy et al., 1988, Ratto et al., 1989, Simkova and Cervenka 1981, Palmateer et al., 1989) we suggest that coliphage tests be included as part of any potable water testing schemes. The coliphage test has an advantage over traditional microbiological tests in that the test can be read after as little as 6 hr., if necessary. It is very economical and simple to perform. The H_2S paper strip test results indicate that this procedure is equally on more sensitive than the TC MPN and MF techniques for testing potable waters, a finding which also was reported by Ratto et al., 1989. Since the H_2S paper strips can be prepared and maintained in sealed bottles for at least 1 year, this would be an ideal procedure for testing isolated water supplies and where laboratory facilities do not exist.

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Table 1. Summary of Coliform and Coliphage Incidence in Three Types of Potable Water Supplies in Thailand

	Coliphage + Coliforms +	Coliphage - Coliforms -	Coliphage - Coliforms +	Coliphage + Coliforms -	Total
Rainwater	14	10	78		103
Deep Wells	5	12	10	10	37
Tap	9	30	25	16	80

Table 2. Summary of Bacteriological and Coliphage Test Data From Deep Wells in Thailand.

Sample	Total Coliform MPN/100mL	Total Coliform MF/100mL	H_2S Paper Strip Test 1-24 hr.	H_2S Paper Strip Test >24 hr.	Coliphage PFU[1]/100mL
D1	<2	0	neg	neg	5
D2	<2	0	neg	neg	5
D3	<2	0	neg	neg	5
D4	2	306	neg	neg	370
D5	<2	0	neg	neg	0
D6	<2	0	neg	neg	5
D7	<2	0	neg	neg	290
D8	<2	0	neg	neg	405
D9	13	0	neg	neg	0
D10	<2	0	+	+	0
D11	<2	0	+	+	0
D12	975	300	+	+	160
D13	<2	0	neg	neg	0
D14	4	140	+	+	0
D15	2400	180	neg	+	270
D16	1	0	+	+	0
D17	5	20	+	+	0
D18	<2	0	+	+	0
D19	11	0	+	+	0
D20	8	380	neg	+	>500
D21	7	0	neg	neg	>500

PFU[1] = plaque forming units