Detection of Microbes in Water Purification Stations and River Water in Japan

S. Yamai¹, M. Ogawa², A. Hase³, H. Morihara⁴, Dr. I. Ideguchi⁵, T. Yasuoka⁶ and H. Yoshikura⁶,

Kanagawa Prefectural Public Health Laboratory¹, Kawasaki City Institute for Public Health², Institute of Environmental Science of Osaka City³, Tottori Prefectural Public Health Laboratory⁴, Public Health Institute of Kochi Prefecture⁵, National Institute of Infectious Diseases, 1-27-1, Toyama-cho, Shinjuku-ku, Tokyo⁶, Japan

Introduction

The drinking water in Japan should satisfy the criteria indicated in Water Quality law of the Ministry of Health number 69 issued at December 21 of 1992. According to the law, the bacterial colony count per 1 ml on the normal agar plate should be less than 100 and that of coliforms per 50 ml should be below the detection level. The detection should follow the procedures appearing in the Annex 1. The water also should satisfy the restrictions regarding 27 toxic chemicals.

The data on the drinking water presented in this document is based on the survey compiled by S. Yamai, Kanagawa Prefectural Public Health laboratory. The survey was performed twice in July and October in 1997.

The data on the river water (November 1996-February 1998) were derived from the reports which were published in Infectious Agents Surveillance Report. The survey was conducted in Kanagawa Prefecture, Yokohama City, Kawasaki City, Shizuoka City, Osaka City and Tottori Prefecture.

Survey of Microbes at the Water Purification Plant

Cryptosporidium outbreak near Tokyo: In June 1996, there was an outbreak of cryptosporidiosis in Ogose-Cho (Saitama Prefecture), a small town near Tokyo. The outbreak was first noticed by a large number of absentees in the primary and secondary schools in the town. It continued about 4 weeks. The investigation on the morbidity was conducted immediately after the end of the outbreak. All the inhabitants in the town were asked to respond whether they had diarrhea during the outbreak. Among 12,345 responses obtained, 8,812 answered yes (71%). Among the individuals with symptoms, 2,878 (32.7%) were unable to work or got to school, and 2,856 (32.4%) visited clinics.

At the beginning of the outbreak, bacterial infections were suspected. However, from the pattern of the outbreak, which affected the inhabitants indiscriminately, the water supply came to be suspected. Cryptosporidium was detected in patients' diarrheal stool specimens and also from the purified water at the water purification station (1,000 protozoa/10 liter).

The water source of the purification plant was the Ogose river. The source of cryptosporidium contamination was traced to two sewage treatment stations which were located very close to the water purification plant (the treated sewage water contained as many as 10,000/10 liter when the epidemic was detected). There was a heavy rain fall four weeks before the onset of the outbreak and flux of the river increased sharply. Several days before the outbreak a sharp peak of turbidity of the water at the purification plant was noticed. Probably, cryptosporidium circulated from men to sewage, then to water supply and to men before the outbreak. The increased water flux may have washed a large number of the protozoa into the...
sewage water and polluted the water heavily with them. Once the water supply was contaminated by cryptosporidium, 6-14 days were needed for clearance of the protozoa present in the water supply system.

The water purification plant used the high-speed filtration method. As the quality of the source water was very high in the previous tests till the previous year, the "purification" depended largely upon the chlorine treatment. As a consequence, the flocculation reagents which should be regularly used for the high speed purification were not regularly introduced into one of the water sources. In addition, as the turbidity meter was not functioning well, the turbidity was monitored only by the naked eye and there was no record of the measurement (1).

Survey of water purification plants for microbial pollutions: Alarmed by the event, microbial contamination of the source water and purified water in 23 water purification plants were conducted (Fig. 1, upper chart).

![Figure 1: The location of the water treatment stations (upper chart) and the rivers (lower chart) where the monitoring of the microbial contamination was conducted.](image)

The methods applied for microbe detection in the survey were the conventional ones, i.e. membrane filtration of the water and culture of the filter-trapped bacteria in appropriate media.

For quantitative estimation of general bacteria, coliforms, fecal coliforms, and fecal Streptococci, 0.01-10 ml water were used as the starting material. For aerobic and anaerobic spore formers, 10-100 ml were used. For Vibrios, Shigella, Salmonella typhi, Enterohemorrhagic...
E. coli O157, Aeromonas, Plesiomonas, Campylobacter, Yersinia enterocolitica, Salmonella species, Legionella, 1 liter water was used. The microbes were detected by the standard culture method. For Cryptosporidium and Giardia, 33 liters were used for water before purification and 16 liter for the water after purification. The protozoa were detected by concentration, staining and microscopic examinations.

As shown in Fig. 2 (data from 18 plants), though present to different degrees in the water before the purification, fecal coliforms and fecal streptococci decreased in number to the acceptable level after treatment. Spore forming bacteria too became undetectable.

Figure 2: Detection of indicator bacteria and pathogenic protozoa in the water before and after purification in 18 water purification plants. The vertical axes indicate number of bacteria per 100 ml or number of protozoa per 33 liter. See upper chart of Fig. 1 for location of plants.

The water was also examined for some pathogens. Among the pathogens searched, V. cholerae O1, Shigella, Salmonella Typhi, EHEC0157, and Yersinia enterocolitica were not...
detected in the reservoirs of untreated water. The pathogens, such as, V. cholerae non-O1, V. fluvialis, A. sobria, Plesiomonas, C. jejuni, C. coli, Salmonella, Legionella, Cryptosporidium, and Girardia (Fig. 3), were detected, however. After treatment, all the pathogens became undetectable. The data suggested that the water in many reservoirs in Japan contained potential pathogens, though water sources, K1, K2, K6, K7 and K8 were less contaminated. The importance of the water treatment was confirmed. It was also suggested that the presently available method could detect many pathogens in the untreated water reserved for the drinking water.

Figure 3: Detection of pathogenic bacteria in the water before and after purification in 18 water purification plants. Closed circles indicate positive detection, and the open circles negative detection. See upper chart of Fig. 1 for location of plants.

Detection of pathogens in the river: Detection of pathogens in the river has been conducted in 6 places (Fig. 1, lower chart). Contamination by various species of Vibrios, such as V. cholerae non-O1, V. cholerae El Tor (CT-), V. parahaemolyticus, V. fluvialis, various species of Salmonella, including S. Enteritidis, S. Typhimurium, S. Paratyphi B, and EPECs 06 and 078 was observed (Fig. 4). In view of a possible contamination of drinking water by the river water or infection through recreational contact, the situation needs attention.

Discussion

The river water and the source water for drinking water in Japan were found heavily contaminated by various pathogens. The situation appears alarming. However, the epidemics related to the water purified by the water purification plants have been rare. Even the large outbreak of cryptosporidiosis, which occurred recently, was largely due to failure of keeping the routine flocculation procedures. Therefore, the present system of water treatment appears functioning well at present in Japan.

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Figure 4: Detection of pathogens in the river water. Closed circles indicate positive detection. See lower chart of Fig. 1 for the location.

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It should be mentioned that, in the above-mentioned outbreak near Tokyo, the source of the pathogen was the nearby sewage treatment stations. As the population grows, more fecal discharge is shed into the water and chance of microbial contamination of drinking water increases. So the situation never allows us complacency. The microbial control of the drinking water will be more in need in future.

Question is whether the drinking water should be monitored constantly for the pathogens or not. The molecular biology technique develops, and it may allow us a real time monitoring of the pathogens. However, we have to consider the following points.

- Stop of the water supply by the water purification plant accompanies a large financial loss. In addition to the financial loss due to failure of selling the water, the plant has to pay a large amount of money for the discharge of the untreated water into the sewage system.

- Detection systems with false positives even at a low frequency are not valid for rare events. Suppose the breakdown of the purification occurs once in two years for a plant and the false positive rate of an assay is 0.2% (99.8% correct). If the monitoring is conducted every day, in 365x2 assays in two years 365x2x0.002 (=1.46) could be false positive. The number of false positives that is 1.46 is higher than one which may actually occur. The assay, which is highly accurate for frequent events, is very inaccurate for the rare events.

Accordingly, the monitoring system has to provide the data which can convince the regulator without any ambiguity, ex. showing the pathogens themselves. The genome detection alone may not be sufficient.

Reference

1. Saitama Prefecture Public Health Administration (1997), Cryptosporidium outbreak - Case report of the diarrheal outbreak in Ogose-cho. Saitama Prefecture Public Health Administration.