FINAL REPORT
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A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water

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ABSTRACT

In 1991, an epidemiological study suggested that 35 percent of gastrointestinal illness could be attributed to drinking water. This generated significant interest from the drinking water community. The potential impact these results led several organizations (the US Environmental Protection Agency, Health and Welfare Canada, the University of Quebec, Compagnie Générale des Eaux, the National Water Research Institute and the AWWA Research Foundation) to contribute to a confirmatory study designed to correct the perceived limitations in the initial research and to confirm or refute its findings. The current study compares the levels of gastrointestinal illness in four randomly selected groups of 250 families, which were served water from one of the following sources:

- Plant effluent water as it leaves the plant (to exclude the distribution system);
- Plant effluent water treated by reverse osmosis [RO] (to eliminate the effect of tap water);
- Tap water (to examine the role of the distribution system); and
- Tap water with a valve on the cold water line (to examine the effect of home plumbing).

The plant was selected for the quality of its operation and the level of contamination of the river water that it treats. While river water was heavily contaminated with parasites, viruses and bacterial pathogens, it is typical of surface water used by many water utilities throughout the world. The plant produced water that met or exceeded current North-American regulations for drinking water quality. The distribution system was in compliance for both coliforms and chlorine.

The rates of gastrointestinal illness among consumers of water obtained directly at the treatment plant were equal or slightly lower than the rate of illness among consumers of water treated by RO. This suggests that current treatment practice at this plant is not an important source of illness. The not-so-good news is that there was an average of 14 percent more illness among tap water consumers than subjects in the RO group, suggesting a potential adverse effect of the distribution system. While this difference was an average for the test period, higher levels were observed in the autumn-winter period of 1993-94 and appear to be the result of two separate events. Children were consistently more affected than adults and up to 40 percent of their gastrointestinal illnesses were attributable to water.

Subjects in the RO and Plant groups still consumed about 30 percent of their drinking water as regular tap water: they were thus exposed to its contaminants and the tap water attributable rates are underestimated. Consumers of water from a continuously running tap had a higher rate of illness than any other group throughout the test period. This was completely unexpected, since the continuously running tap was thought to eliminate the effects of regrowth in home plumbing.

Although there are several unsubstantiated theories as to the cause of this effect, it remains unexplained but suggests an effect related to the distribution system.

This project has attained two of its objectives: determining the level of tapwater-related gastrointestinal illnesses and identifying the source of these illnesses. We have however failed to identify any potential indicator for these health effects, especially in view of the fact that they appear to be related to the distribution system. Potential follow-up research would examine which parameters contribute to the apparent effect in the distribution system and investigate the role of the continuously running tap in the occurrence of gastrointestinal illness.

1. INTRODUCTION

1.1 RESEARCH GOALS

- The primary objective of this project was to evaluate drinking water related gastrointestinal illnesses in order to confirm (or refute) observations made previously in a population consuming tap water meeting current water regulations.

- The second objective, should health effects be observed, was to determine the source of these illnesses such as inadequate treatment, regrowth in distribution system or household plumbing, etc.

- The third objective was to provide Regulatory Agencies and public health authorities with information on health risks associated with drinking water and, if any, to find suitable indicators for these health effects.

1.2 PROJECT BACKGROUND

Drinking water treated to today's standard is not an important source of mortality as it was at the beginning of the century. Mortality due to typhoid fever is now a very rare occurrence and most of this reduction was achieved through chlorination of drinking water, pasteurization of milk, several public health measures and better sanitary conditions. Several outbreaks of gastroenteritis and hepatitis (Bloch *et al.* 1990, Wilson *et al.* 1982), giardiasis and cryptosporidiosis (Smith and Smith 1990, Hayes *et al.* 1989, Smith *et al.* 1989, Kramer et al. 1996, Morris et al. 1996) in communities with water meeting current regulations, have brought to the public attention the fact that the coliform standard (i.e. absence of fecal coliforms and minimal numbers of total coliforms) may not be as efficient as expected.

Microorganisms most frequently associated with waterborne outbreaks are bacterial (*Shigella* and *Salmonella*), parasitic (*Giardia lamblia* and *Cryptosporidium parvum*) and viral (rotaviruses and Norwalk). Bacterial outbreaks have been generally associated with failures of treatment or contamination of the distribution system. On the contrary, parasitic and viral outbreaks have been often associated with water that appeared properly treated and free of current indicators of contamination. In the United States, the review of waterborne outbreaks indicates that about 50% of these outbreaks were characterized by acute gastrointestinal symptoms of unknown etiology (Craun 1990).

The fact that water meeting current standards is not always safe and the fact that there has been an increase in the number of reported outbreaks might be explained by several facts:

- a better reporting/surveillance system (subject to variations due to funding),
- an increased demand on water treatments plants (deterioration of raw water),
- aging of the treatment plants (with occasional failures),
- a higher level of hygiene which restricts circulation of microorganisms,
- a decrease in the immunity level to these microorganisms.

The correct answer lies probably in a combination of all these factors, but the fact remains that public health is threatened if the current indicators of water quality are not as adequate as they appear to be.

While some infectious diseases have been shown to be transmissible by water (e.g. hepatitis A, *Salmonella*, *Giardia*), very little is known on the number cases associated with water. Some data have been collected gastrointestinal illnesses especially in outbreak situation, but almost nothing is known for non–gastrointestinal diseases associated with water. Hepatitis, poliomyelitis, viral meningitis, enteroviral carditis, epidemic myalgia, diabetes are all virus–induced illnesses other than gastrointestinal that could be waterborne. The estimation of the waterborne fraction of most diseases is impaired by their low level in the population. Cancer is a chronic longer term issue and events occur annually in very few individuals. Hepatitis, aseptic meningitis and other viral diseases also occur relatively rarely. On the contrary, gastrointestinal symptoms offer a frequent and easily measured index of health effects: diarrhea or vomiting occur in most normal individuals once every two to three years. The probability is high that a large proportion of the population will experience had an episode of gastrointestinal illness, water–related or not. We have relied on this normally high rate of disease to attempt to evaluate the waterborne fraction of these diseases (Payment et al. 1991b).

1.2.1 Health effects of drinking water

The estimated life-time probability of infection due to low levels of virus and parasites in drinking water are predicted to be quite high (Regli *et al.* 1991). Models and the observation that many enteric microorganisms produce only mild infections, might explain the current level endemic of gastrointestinal illnesses and why many enteric outbreaks are classified as being of "non-bacterial etiology" (Rotbart 1995, Payment 1993a, 1993b).

In the absence of evident acute health effects (i.e. epidemics or outbreaks), epidemiological studies have been centered on long term effects of potentially carcinogenic chemicals (Crump and Guess 1982). Health effects of disinfection by-products have also been extensively studied but have remained inconclusive (Riley 1995, Morris *et al.* 1992). Birth defects or abortions have also been associated with the consumption of tap water even though much of this effect might have been due to recall bias during retrospective and matched controls studies (Swan *et al.* 1992).

Batik *et al.* (1979), using Hepatitis A virus cases as an indicator could not establish a correlation with water quality in the United States and found no correlation between current indicators and the risk of waterborne outbreaks (Batik *et al.* 1983).

In France, Collin *et al.* (1981) prospectively studied the gastrointestinal illnesses associated with the consumption of substandard tap water using reports from physicians, pharmacists and teachers. Their results were based on more than 200 distribution systems of treated or untreated water: they reported five outbreaks (more than 1000 cases) associated with poor quality water. In most of these studies the researchers relied mainly on the detection of outbreaks to assess the level of water quality and they did not address the endemic level of gastrointestinal illnesses such as the one that would result from low level contamination of the water.

Recent data from the Milwaukee outbreak in the United States, has suggested that the outbreak was associated with an increase in turbidity at one of the two water plants of this city (Morris et al. 1996). In Israel, Fattal *et al.* (1988) addressed the health effects of both drinking water and aerosols from wastewater treatment plants. Their data on kibbutz water quality and morbidity was performed in an area with relatively high endemicity of gastrointestinal disease and did not show a relationship between health effects and total or fecal coliforms. This study was based on morbidity reported to physicians. In Windhoek (Namibia) Isaäckson and Sayed (1988) conducted a similar study over several years on thousands of individuals served by recycled waste water or normal drinking water. They did not observe an increased risk of reported acute gastrointestinal illness associated with the consumption of recycled waters. The populations compared had higher incidence rates than those observed in North America and they were subjected to a high exposure level due to other sources thus masking low levels of illnesses.

1.2.2 Previous studies by our group

Our previous project (Payment *et al.* 1991a, 1991b) was intended to directly and empirically measure the level of gastrointestinal (GI) illness related to the consumption of tap water prepared from sewage-contaminated surface waters and meeting current water quality criteria. A randomized intervention trial was carried out; 299 eligible households were supplied with domestic water filters

(reverse-osmosis) which eliminates microbial and chemical contaminants from their water and 307 households were left with their usual tap water without filter. The GI symptomatology was evaluated by means of a family health diary maintained prospectively by all study families over a 15-month period.

The estimated annual incidence of GI illness was 0.76 among tap water drinkers as compared with 0.50 among filtered water drinkers ($p<0.01$). These findings were consistently observed in all population subgroups (Table 1). It was estimated that at least 35% of the reported GI illnesses among the tap water drinkers was water-related and preventable. The remaining illnesses were probably attributable to other endemic illnesses, food-related infections or allergies. summarizes data for the two groups of families under surveillance from February 1989 until June 1990.

Table 1 Incidence of highly credible episodes of gastrointestinal illnesses during a prospective epidemiological study of water related illnesses in 600 families (Payment et al. 1991a)

	RO-Filtered water			Tap water			
Age group	n	Rate	S.E.	n	Rate	S.E.	Relative risk
0 - 5	115	1.13	0.18	72	1.60	0.27	1.42
6 - 20	428	0.46	0.07	427	0.66	0.08	1.43
21 - 49	512	0.40	0.06	458	0.57	0.08	1.43
50+	78	0.23	0.12	126	0.33	0.11	1.43
All	1133	0.48	0.06	1083	0.65	0.07	1.35

Rate = Annual incidence per person
S.E. = Standard error corrected for cluster sampling
Relative risk when compared to RO group

The rate of disease increased with the amount of water consumed (i.e., a dose-response effect was demonstrable). There was no correlation between the *number of episodes* and total or fecal coliforms, chlorine or heterotrophic bacteria in the tap water.

There was an association between the *duration of the illnesses* and HPC bacteria at 20°C (Payment *et al.* 1993). However, due to the large number of analyses performed this could have been a spurious result due to chance. In the filtered water consumer group, there was a significant correlation between the rate of disease and the HPC bacteria growing at 35°C on R2A medium but no relationship with the amount of water consumed (Payment *et al.* 1991b).

The rate of water-related diseases increaed with distance from the plant (Figure 1) and bacterial regrowth was suggested as an explanation. Studies on the virulence of bacteria isolated from tap water revealed that a small fraction of these bacteria could be considered as potential pathogens (Payment *et al.* 1994) and it was suggested that their multiplication to significant numbers could be a health risk (Payment 1995).

Attempts were also made to determine the etiology of the observed illnesses. There was no indication by serology of water-related infections by enteroviruses, hepatitis A virus, rotavirus (Payment 1991c) or Norwalk virus infections (Payment *et al.* 1994).

The societal cost of these "mild illnesses" could be several orders of magnitude higher than the costs associated with acute hospitalized cases. In the US, it was estimated that the annual cost to society of gastrointestinal infectious illnesses is *$19500 million dollars for cases with no consultation by physician*, $2750 million dollars for those with consultations, and only $760 million dollars for those requiring hospitalization (Garthright *et al.* 1988, Roberts and Foegeling 1991). These estimates do not even address the deaths associated with these illnesses particularly in children and older adults.

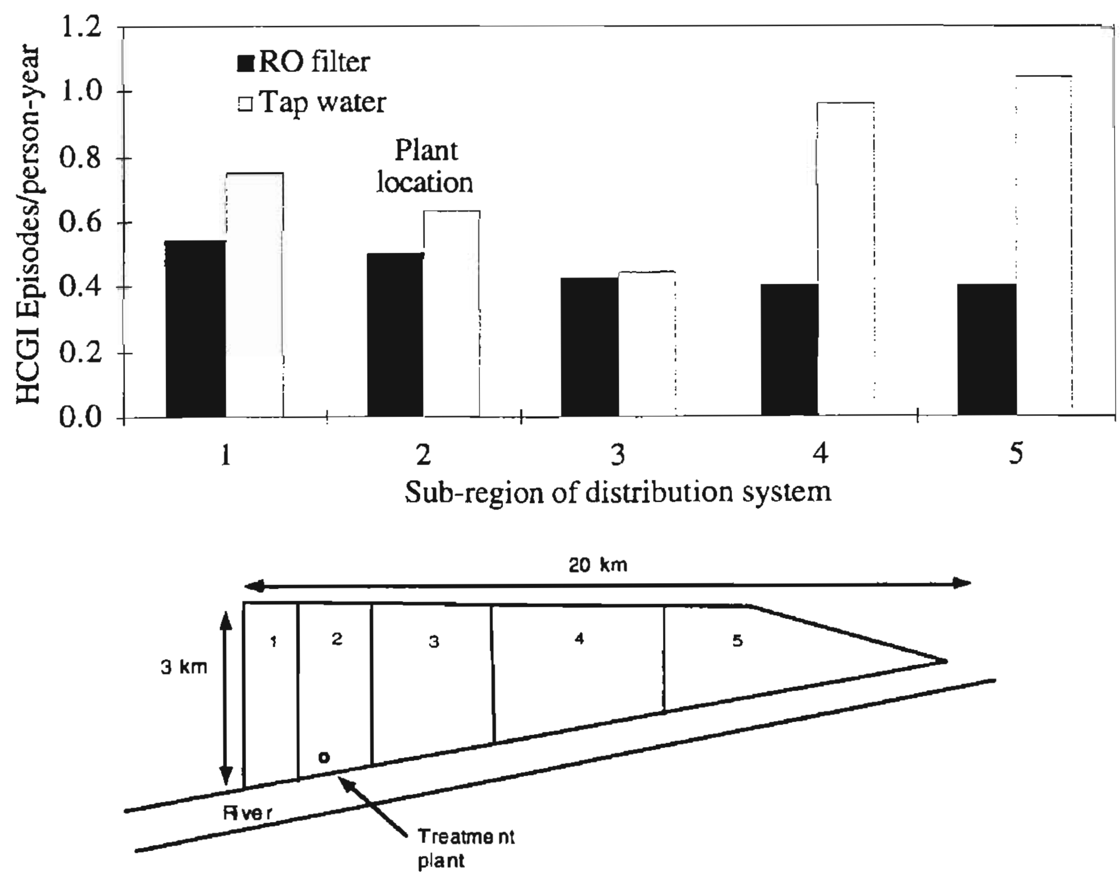


Figure 1 Incidence of highly credible episodes of gastrointestinal illnesses: effect of distance from water treatment plant (Payment et al. 1991.

2. RESEARCH METHODOLOGY

2.1 GENERAL DESIGN

The objective of the current study was to reevaluate the level of gastrointestinal illnesses attributable to tap water in a population consuming drinking water meeting current regulations and to determine the source of these illnesses.

A randomized intervention trial was carried out in an area served by a single water treatment plant and its distribution system. We constituted 4 study groups composed of randomly assigned families with at least one children 2 to 12 years old. Gastrointestinal and respiratory symptoms of the subjects were recorded from September 1993 or from the time of their enrollment in the study, until December 1994.

The four groups of randomly selected and assigned subjects were requested to drink respectively:

1. Tap water (Tap group)
2. Tap water (with a bleeder device installed on the cold water line under the kitchen sink) (Tap-valve group)
3. Water obtained at the treatment plant and bottled (Plant group)
4. Water obtained at the treatment plant, treated by reverse-osmosis and bottled (RO group)

Tap: this group was designed to establish a baseline value of the level of illness in the population under surveillance as the incidence of gastrointestinal and respiratory illnesses is variable over time in a given population, in different geographical areas as well as in subpopulations of the same area. It can range from as low as 0.2 episode/person-year and as high as 5 or more episode/person-year. This group thus served as the baseline from which we could estimate the variation of the level of gastrointestinal illnesses in the population under surveillance.

Tap-valve: in these households, a bleeder valve was installed under the sink of the kitchen. This valve permitted water to flow continuously to the drain. This procedure was used to establish the contribution to the illness rate of the service line and the household plumbing. It was hypothesized that, by maintaining a constant flow of water, the water consumed by the subjects would be equivalent to water in the distribution system thus estimating the contribution of the distribution system to the illness rate.

The subjects in the two following groups received bottled water. This was intended to achieve some level of blindness as subjects would not know what type of water they consumed and to assess the contribution of the water treatment plant.

Plant: subjects in this group were given bottled tap water obtained directly at the water treatment plant. It was unmodified by the distribution system and thus, was intended to assess the contribution of the distribution system to any health effect. It was bottled in unmarked containers and delivered to the subjects.

Reverse-osmosis (RO): subjects in this group were given water from the treatment plant that was further treated by reverse-osmosis. It was also bottled and constituted one way to repeat our the earlier study in which each household had been fitted with RO domestic filters. Because, a reverse-osmosis unit could not be obtained early in the study, bottled spring water was used as a temporary replacement.

2.2 STUDY AREA

Selection of the area was the subject of long discussions. Repeating the study at another site would have raised more questions than answers and it was agreed by the sponsoring agencies that a repeated study, enhanced to answer questions raised by the first study, would be preferable both scientifically and economically.

The population selected is served by a single water treatment plant and is located in the same area studied earlier (Payment *et al.* 1991a). The area is a suburb of average socio-economic level with few industries. A portion of the close-by territory is still agricultural, but the area under study is well urbanized and typical of suburbs that have developed in the last 50 years. The main residential type is the bungalow, but in certain areas 2 or 3 story high apartments building are common. The area is about 3 km by 20 km long (see Figure 1).

2.3 WATERSHED AND TREATMENT PLANT

The original choice of the water plant was made on the two following criteria:

1) a polluted raw water source (i.e., presence of pathogens, especially viruses and parasites),
2) a plant that was known to be operated according to standards that would be recognized by Canadian, American and international authorities.

The watershed is similar to many American rivers to which it compares in terms of microbial pollution, including parasites and viruses (LeChevallier et al. 1991, 1995, Rose et al. 1991, Gerba and Rose 1990, Payment and Armon 1989). The main drainage basin is 146,000 km2 and the mean flow is about 2000 m3/sec. The drainage basin includes several large urban centers located about 200 km upstream: there are four municipalities with populations over 25,000, some pulp and paper mills, inorganic chemical plants and metallurgical plants. A large lake located upflow is of slightly better bacteriological quality and is used for recreative purposes. Water quality deteriorates as it enters the river of interest. The area is highly urbanized and typical of many suburban areas. Very little industrial or agricultural discharges are observed within 25 km. Sanitary and storm water collectors are present on both sides of the river. Discharges from combined sewers overflows occur during heavy precipitation.

A schematic plan of the treatment plant is presented in Figure 2. The capacity of the plant is 100,000 cubic meters per day. Preoxidation with ozone or chlorine is possible, but was not used during the course of the study. Water is first flocculated using alum and activated silica and settled dynamically (4 superpulsator and 4 regular). There are 15 rapid sand filter beds and filtration is achieved on dual media filters composed of sand and anthracite, and 2 activated charcoal filters. After filtration, water is ozonated and a final disinfection using chlorine is applied.

Tracer experiments using fluoride were performed to assess contact times for the oxidants.

Valved connections for obtaining treated water as it leaves the plant were put in place in one of the high pressure mains from the clearwell. Sanitary connections were made to a reverse osmosis unit and to sanitary tubing for transfer of plant or RO-treated plant water to a sanitary tanker truck. All connections were protected with sanitary caps when unused.

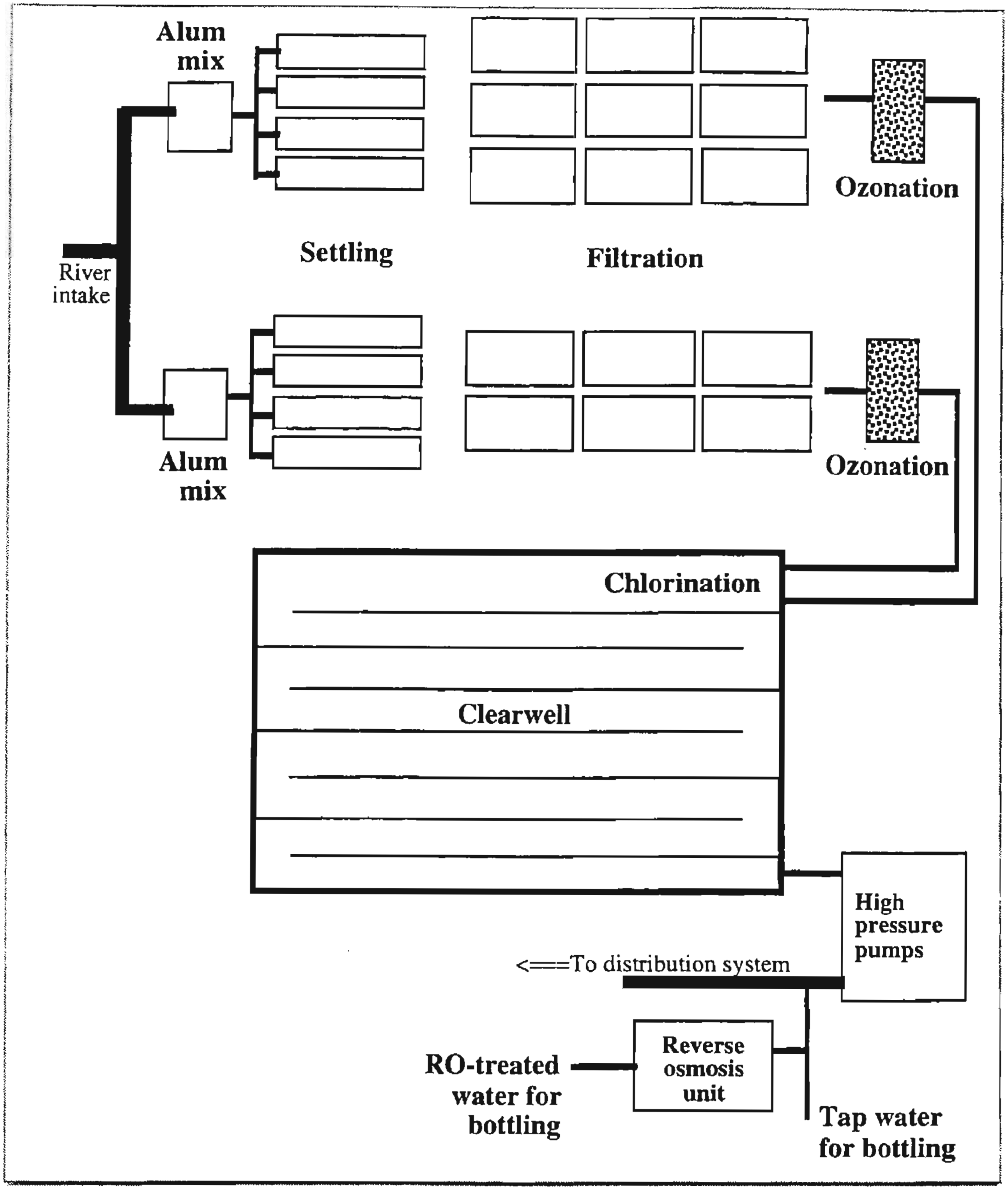


Figure 2 Schematic description of water treatment plant with connections to reverse osmosis unit and plant water.

2.4 WATER DISTRIBUTION SYSTEM

The water distribution system is composed of : cast iron, cement lined gray iron, iron and cement. There are no reservoirs in the system and water is supplied on demand. The different sizes of pipes in this system are presented in the following table:

Table 2. Distribution system characteristics: diameter and length of pipes.

Pipe diameter		Pipe length
inches	mm	meters
48	1220	573
42	1067	2480
36	914	1547
30	762	3201
24	610	8023
20	508	3002
18	457	586
16	406	27152
14	356	9929
12	305	40476
10	250	25450
8	205	69764
6	152	176115
4	102	3446
3	76	596
2	50	590

2.5 REVERSE-OSMOSIS UNIT

A reverse-osmosis unit was leased from Airableau Inc. (Canada). It was rated at a product flow rate of 120 L/min (30 gpm) at 4°C. The unit was composed of two sets of ten modules (10 cm diameter) Hydronautics Membranes. It was installed at the water treatment plant close to the water main and was used from February 1994 to supply RO-treated tap water. The unit was connected through sanitary tubing to water. Shut-off valves and sanitary rapid connections were provided and all connections remained covered with sanitary caps between uses. The efficiency of the unit was monitored on-line by conductivity and pressure controls and samples were taken at each use for bacteriological analysis of the product water.

2.6 WATER CONSUMED BY EACH GROUP

2.6.1 Tap group

Participating families in this group consumed on a regular basis tap water from the water treatment plant distribution system. The water was not modified and the subjects did not change their normal consumption pattern.

2.6.2 Tap-valve group

To evaluate if bacterial regrowth in household plumbing plays a role in gastrointestinal health effects, purge valves were installed by professional plumbers in a randomly chosen sub-group of households. These valves maintained a sufficient flow rate to the drain to equilibrate the water quality in the household with the water quality in the main. The derivation valve was installed on the cold water line under the kitchen sink with a drain to the sewer line. The valve was normally installed less 60 cm (24 inches) from the tap with back-flow control valves and according to local building codes. Two types of valves and tubing were installed. The first one was composed of 1/2 inch (12 mm) tubing, a T, and a ball valve to control the amount of water that was rejected to the drain. Both copper (no-lead solder) and PVC tubing were used. The second type was used when difficulties were encountered during installation. It was composed of a strap-on system with a self-tapping needle valve connected to 3/8 inch copper tubing derived to the drain. This system allows water to flow at a rate of 100 to 500 ml/min. and was adjusted to minimize noise due to the continuously running water.

2.6.3 Plant group (bottled water)

A commercial bottling company was selected for the study. The facility is located 100 km from the water treatment plant and a sanitary stainless steel tanker truck was used to haul the water. Because the underground aquifer used by this company is located far from the bottling facility, their tanker truck is used exclusively to haul spring water from the pumping site of the aquifer to the bottling facility. It was used to transport our treated waters to be bottled.

The normal sequence for cleaning the tanker was: a wash cycle with detergent, disinfection with chlorine bleach, rinsing with tap water from the city where the bottling facility is located. It was then used to haul water used by the bottler in its normal activities. The RO-unit was left to run at full flow-rate for 15 minutes before being connected to the tanker truck inlet. The truck loaded 20,000 liters of water and hauled it to the bottling facility. Water was collected at the water treatment plant every other Thursday, bottled the following day and delivered on the next Monday morning to a warehouse. Occasionally, it was collected on Monday morning, bottled and delivered on the same day to the warehouse.

Two sequences of bottling were used. If the water was collected later during the day, it was left overnight before bottling. To reduce taste and odor problems associated with plant water, it was aerated overnight using a bubbling apparatus connected to a sanitary compressor. If the water was collected early in the day, connections are made for direct transfer of the water to a large tank. In both cases, the water was pumped using a sanitary pump and tubing. This tubing was also fitted with a Venturi injector with which oxygen was injected to further reduce taste and odors.

Water was bottled in standard 4-liter plastic containers. Each bottle was unmarked except for a code that included the date, the time of bottling, the work shift, and our own code. Immediately after bottling, bottles were packaged in cases of four which were delivered to a warehouse at the university. It was kept there until picked-up for delivery during the following 2 to 4 days.

Water from the plant was found to have a strong metallic and chlorine taste and odor: some subjects refused to drink this water. The problem was corrected by aerating the water overnight using air diffusers in the truck and oxygenating the water at the time of bottling using a Venturi-type injector.

2.6.4 Reverse-osmosis (RO) group

The effect that was sought in the RO-treated water group was to expose the population to water that was essentially free of the contaminants that could still be present in the tap water. This group was supposed to receive RO-treated water from the treatment plant. RO-treated water prepared from tap water at the water treatment plant was similar to our original study in which individual domestic RO units were used.

Because the RO-unit was not available until December 1993 and as a temporary measure, we used spring water of high quality obtained from the company selected to bottle water. The spring water used was of recognized high quality and has never been reported to be fecally or otherwise contaminated (results available from the Ministry of Environment of Quebec). Total bacterial counts were less than 10 cfu/ml. Water is obtained from protected aquifer, and is ozonated at the time of bottling. The aquifer is 21 meters (70 feet) deep and water is pumped through a well that is protected by a small sheltered building kept under lock.

Spring water was delivered to the subjects in RO-group until February 1994.

The single large RO unit has the advantage of providing a single water quality to all subjects and to prevent bacterial regrowth in the storage reservoirs of the domestic units. Finding an adequate system at reasonable cost was difficult.

Water was collected, bottled and delivered as described for Plant bottled water (section 2.6.3)

2.6.4.1 Water delivery to subjects

Water was delivered every two weeks to the subjects by a local delivery company. Bottled water was picked-up at a warehouse and delivered to the subjects on the week it was bottled.

Subjects were instructed to use this water for the following 2 weeks. Subjects were requested to discard the water left over from the previous delivery when freshly bottled water was delivered to them.

Enough water was provided to each family for a two-week period. If they did not have sufficient water during this 2-week period, they could call our offices and obtain supplementary bottles from the same production lot.

2.7 EPIDEMIOLOGICAL METHODS

2.7.1 Population evaluated

The study was planned to include 4 groups of 350 families followed for 16 months (September 1993 to December 1994). The purpose of the study was not to evaluate special risk groups at such as immunosuppressed, immunocompromised, elderly, etc., thus the individuals selected were in good health with no known medical conditions that may affect the outcome. The study was carried out in a suburban area of average socio-economic level. Apart from the fact that most of the population of this area is French-speaking, it is quite typical of an average socioeconomic level, the subjects were in good health and should reflect an average North American community.

2.7.2 Selection process

The study design called for the constitution of four groups of families:

1. Normal use of tap water;
2. Normal use of tap water plus an under the sink purge valve;
3. Use of plant effluent water (bottled);
4. Use of plant effluent water treated by reverse osmosis (bottled).

Four eligibility criteria were established for participation in the study:

1. Located within zone of influence of the water filtration plant;
2. Usual consumer of tap water supplied by the city;
3. At least one child aged 2 - 12 years living full-time in the household;
4. Able to communicate in French.

The sampling frame was the list of families residents in the study area and benefiting from a government-run income supplement program for families with children under 18 years. The government agency responsible for the program sent us a list of families residing in the area.

Based on the response rates and percent of eligible families obtained in the 1988 study, we requested a first random sample of 3200 names. Because the number of eligible families was lower than expected, a second request was presented later to obtain the remaining 1600 names. These were randomized unequally to each group to maintain groups of roughly equal size.

Families were first contacted by mail to inform them of the study and that an interviewer would be calling them within a few days. Within the next week, telephone interviewers contacted the family to assess eligibility. If the family satisfied the eligibility criteria and agreed to participate in the study, it was assigned randomly to one of the four groups. This process of screening and random

allocation was continued until there were approximately 350 households per group. This number of families was chosen in the hope that by the end of the follow-up period while there would remain at least 250 families per group.

While subjects knew from the introductory letter that there would be four groups, they were given no choice as to the group they could participate. They were however always given the possibility to refuse to participate at any step of the selection process as well as during the observation period.

Interviewers visited the families to explain the study and to obtain written consent forms from all members of the family that agreed to participate, answer basic questions and explain to them the Health Watch Calendar. Further sociodemographic and water consumption data were obtained on two other occasions, in June and December 1994, from self-completed questionnaires which were returned to us by mail. The baseline questionnaire included sociodemographic information, as well as information on place of work, school or day-care attendance, trips, chronic medical conditions and use of medication. Arrangements were made with each family to agree on a convenient time for delivery of the water if they were in the bottled water groups. For those in the tap-valve group, we made arrangements for a plumber to install the valve at the kitchen tap.

2.7.3 Health survey

2.7.3.1 Survey calendar and incentives

A simple diary-type questionnaire with a list of symptoms was distributed to all subjects and a responsible parent assigned to record DAILY symptoms experienced by members of the family (see Annex 1). The Calendar was distributed as a spiral bound booklet 8.5 x 11 inches containing *an "Introductory Letter"* and all the two-week questionnaires dated for the observation periods. Each record sheet was printed on both sides and could be used to record the events of 4 individuals during each two-week period. If there were more than 4 subjects in the household, a second booklet was provided.

The subjects were asked to record the requested information ***on the day it occurred***. Every two weeks, families were contacted by telephone to obtain the information recorded on the diary. The interviewer was only required to record the information already on the daily diary. As most weekly reports were negative and the information sought fairly simple, telephone calls were relatively short. The interviewer asked if any of the subjects had experienced cold or flu, vomiting, diarrhea or nausea. If any these symptoms was reported, the interviewer requested the complementary information recorded on the questionnaire (i.e., all other symptoms experienced). The interviewer

also asked what was the perception of the family of the water quality during the period. School or day-care attendance, trips out of the country or absence from home were also noted on this diary.

To minimize information bias, each interviewer received alternately a different subgroup of subjects to contact. To help maintain the level of participation and maximize contact with the participating families, a lottery ticket was sent to each family every month with a short note thanking them for their continued participation.

2.7.3.2 Water consumption surveys

Three water consumption surveys were done during the course of the study. They were included in the sociodemographic surveys which were done at the time of enrollment, in June 1994 and in Autumn 1994. The questionnaire used was designed to establish a rough estimate of the consumption of different types of water and where it was consumed. It was important to differentiate unmodified tap or bottled water from water that was used for preparing tea, coffee or food (see Annex). The first questionnaire was supposed to establish a baseline consumption of the subjects before they entered the study. However, delays in completing the questionnaires by the subjects resulted in obtaining some results after they had terminated their participation in the study. The data from this first questionnaire, while still useful, cannot serve as a pre-study baseline.

2.7.3.3 Saliva and serum samples

Saliva and serum specimens were collected and archived (-35°C) to provide a source of antibodies for further testing should health effects be observed. These samples could serve to identify the etiologic agent(s). Volunteer subjects were asked by the visiting nurse to sign a consent form on each visit.

Saliva samples were collected during the initial interview and from December 1993, upon report of vomiting, liquid diarrhea or significant illness. The visit to the households were done a maximum of three weeks after the episode: the information being obtained at the time of the telephone interview (1 to 14 days since the illness), and a few days were required to arrange for a visit of the nurse to the subject's home. Each subject was requested to chew on sugarless gum for 30 to 60 seconds to promote saliva production. The saliva was collected by spitting in a beaker and transferring to tubes identified by the subject's name. Specimens were brought back to the laboratory, the volume of sample and identification were recorded and the samples were archived by freezing at -20°C. The information was then recorded in the database keyed to each individual.

Blood samples were collected by venipuncture from a sub-group of saliva-contributing subjects to later validate saliva samples if required. Subjects were asked by the visiting nurse to sign a consent form on each occasion. Serum was separated from the blood and was archived at -20°C. The information was then recorded in a database keyed to each individual.

Saliva and blood samples were collected from volunteer subjects. A total of 5200 saliva samples and 212 serum samples were collected. Several samples were collected in the early stages of the study before any report of symptoms and could be useful to establish baseline data for any disease. Further samples were obtained upon report of vomiting or liquid diarrhea by the subjects. On these occasions the visiting nurse also obtained samples from non-ill family members.

2.7.4 Public health response team

A public health response team was put in place to respond to the detection of an abnormal situation such as a significant increase in the rate of reported gastrointestinal illnesses in any of the groups. The Public Health Department (Régie régionale des services sociaux du Québec) appointed a team of physicians to the researchers.

2.7.5 Data entry and analysis

The database was prepared using FileMaker Pro for the Macintosh. All data on each member of the participating families were entered in this database, verified and validated. Discrepancies were resolved. Data entered in the database were further cross checked and re-verified to ensure its accuracy. All data were first analyzed using SAS Software and SPSS Software installed on a SUN Microstation and stratified according to the variables entered.

For analysis purposes two periods were distinguished: the 1993-94 school year from September 1993 until June 1994 and the remaining months from July 1994 to December 1994. In the Province of Quebec, the end of June marks the end of the school year as well as the period in which a relatively large proportion of the population moves from one household or apartment to another.

Highly credible gastrointestinal (HCGI) episodes were defined as: 1) vomiting or liquid diarrhea alone, or 2) soft stools or nausea with abdominal cramps. This is the same definition that was used during our initial study (Payment *et al.* 1991a). The time criteria for inclusion in the analysis and acceptance as a credible episode was a period of at least 6 consecutive symptom-free days between the episodes. Symptom occurrence and HCGI rates were calculated for each group and stratified according to the collected variables. Estimates of incidence of HCGI illnesses in the groups were derived by means of Poisson regression methods (Frome 1983). Since this technique counts each

episode as an independent event, a correction had to be made for the correlation between repeat episodes by the same unit of observation. This was done using the 'quasi-likelihood' approach (McCullagh and Nelder 1983), which amounts to reducing the nominal significance of each result by a factor related to the amount of this correlation.

Some analyses were based on the entire family as the unit of observation, while others were based on selected individuals within the family. In addition to group membership, some covariates were included in the corresponding models in order to adjust for any imbalance in the groups. These covariates were: age, sex, and subregion. When the analyses were carried out for the entire period of follow-up, the study period was also incorporated as a covariate in the model. Adjusted rates were estimated from the results of the fitted models. Each regression model gave an estimate of the p-value associated with group membership.

Poisson regression was also used to establish whether there was a dose-response relationship between tap water consumption and incidence of HCGI in the tap and tap-valve groups. This was done by adding the number of glasses of water consumed per week as an independent variable to the models. These analyses were based on unmodified tap water, i.e., water other that used in food preparation or cold and hot beverages.

2.8 PHYSICO-CHEMICAL ANALYSES

All physico-chemical analyses were performed at the water treatment plant laboratories according to Standard Methods. Total coliforms, HPC, alkalinity, hardness and color were monitored twice a day at the plant. Turbidity, pH, flow, pressure, temperature and disinfectant residual were monitored continuously at the water treatment plant.

Distribution system events (repairs, breaks, flushing, etc.) were also recorded and are available.

2.9 MICROBIOLOGICAL ANALYSES

2.9.1 Bacteriological analyses

2.9.1.1 Sampling

Samples were obtained from a tap at the water treatment plant, from selected taps in the distribution system and from bottled water. Samples were collected from taps using 100 ml bottles and standard procedures. Chlorinated water samples were collected in bottles containing sodium thiosulfate. Compliance monitoring data for the distribution system were obtained from the Water Utility authorities from analysis of samples collected at selected consumers taps.

2.9.1.2 Tap water and distribution system

Heterotrophic plate counts (HPC) on R2A medium at 20 and 35°C, coliforms on m-Endo, *Aeromonas* on Ryan medium, direct counts and viable counts were monitored in collaboration with researchers from the École Polytechnique (University of Montreal) under an AWWARF contract. With the exception of *Clostridium perfringens*, bacteriological analyses were performed according to standard methods using membrane filtration equipment and the indicated medium (Standard Methods 1992).

The enumeration of *Clostridium perfringens* was performed using the membrane filtration method on m-CP medium described by Bisson and Cabelli (1979) as modified by Armon and Payment (1988).

2.9.1.3 Bottled water

Three to six bottles of each lot of bottled water were analyzed for bacteriological parameters required by the Québec regulations: heterotrophic plate counts at 20°C and 35°C on R2A medium, coliforms on m-Endo medium, *Aeromonas hydrophila* (on Ryan medium), *Pseudomonas aeruginosa* (on m-PAc medium).

Three set of analyses were performed on each lot of bottled water: one at the time of collection, one upon delivery to our warehouse, and one after 2 weeks at room temperature to simulate storage by the subjects.

2.9.2 Virological and parasitological analyses

Large volumes water samples from the water filtration plant were analyzed every two weeks. A raw water sample was normally collected on Monday, while filtered and finished water samples were collected on Tuesday. This schedule was established because of time constraints for obtaining these samples.

Raw (100 liters), filtered (1000 liters) and finished water (1000 liters) samples were analyzed for the presence of human enteric viruses and parasites according to a single method developed in our laboratories. This was essentially a filtration method on electronegative wound fiberglass cartridges (Payment *et al.* 1989). Water was conditioned to 0.001M aluminum chloride and pH 3.5 with 1N hydrochloric acid using an in-line injection system. Sample pH was monitored by an in-line pH meter. Filtration was performed using two 1 µm electronegative Diamond Filter Tubes cartridge placed in series (Filterite Corp., Timonium, MD).

Microorganisms were eluted using 1.8 liters of a 1.5% solution of beef extract (DIFCO, powder) pH 9 and 1% Tween 80. Elution was performed as a slow back-wash with agitation and the eluate was collected in a container with a few drops of Antifoam B. The eluate was then adjusted to pH 7 using 1N HCl, centrifuged at 3000 x g for 15 min. and the pellet resuspended to 40 ml with 10% formalin. This pellet was used for parasite detection. The supernatant was divided in two parts: 1000 ml were used for human enteric viruses enumeration and the remaining 800 ml were used for the enumeration of bacteriophages.

The efficiency of the method was 78% for the human enteric viruses, 77% for coliphages, 52% for *Giardia* and 44% for *Cryptosporidium* (Payment *et al.* 1989).

Human enteric viruses contained in 1000 ml of eluate were reconcentrated to 50 ml by organic flocculation at pH 3.5 using ferric chloride as a flocculating aid. Concentrates were stored at -70°C until assayed. Before inoculation to cell culture the concentrates were centrifuged at 3000 x g for 30 minutes to remove debris. Samples were assayed on MA-104 cells using a maximum of 1 ml of inoculum per 25 cm^2 of confluent monolayers. For each sample or dilution, 10 flasks were inoculated. Flasks were incubated at 37°C for 7 to 10 days, frozen at -20°C, thawed, re-inoculated on the same cell line in 24-well plates and incubated for another 10 days. Monolayers from the second passage were fixed and submitted to an immunoperoxidase immunoassay using human

immune serum globulin. This method detects cultivable human enteric viruses and uses human immune serum globulin (HISG) for the detection of these viruses. The number of viruses in the sample was assessed using the number of wells showing cytopathic effect or immunoperoxidase positive cells after the second passage and was calculated using an mpn calculation programmed in FORTRAN for Macintosh. The results were expressed as the most probable number of infectious units per liter (mpniu/L) (cytopathic effect positive wells and/or immunoperoxidase positive wells).

Somatic coliphages contained in 800 ml of eluate were reconcentrated using a second-step floating layer ammonium sulfate method (Armon *et al.* 1988). Bacteriophages were collected in the floating layer formed when the eluate, containing detergent, was mixed with saturated ammonium sulfate. The floating layer was solubilized in a small volume of distilled water (final volume 25 ml) and assayed. Bacteriophages were assayed by the double layer technique at 37°C. The host for somatic coliphages was a clone of *E. coli* C (ATCC 13706) selected in our laboratory for its resistance to nalidixic acid. It was identified as strain CN-13 and was grown in nutrient agar with 0.01% nalidixic acid.

Parasites were recovered by centrifugation at 3000 x g for 10 minutes from the eluates obtained from large volume sampling and purified by Percoll-sucrose density gradient flotation. Parasites were collected on cellulose acetate membranes and identified by specific immunofluorescent methods using an anti-*Giardia* and an anti-*Cryptosporidium* monoclonal antibody (Hydrofluor, Meridian Diagnostic, Cincinnati, Ohio). Identification of *Cryptosporidium* and *Giardia* cysts was based on a bright green fluorescence and appropriate size (*Giardia*: oval 10-20 x 5-15 µm; *Cryptosporidium*: spherical 5 µm).

3. RESULTS AND DISCUSSION

3.1 RECRUITMENT AND ENROLLMENT

The same approach that was used in 1988 to enroll families was used (Payment *et al.* 1991). An introductory letter was mailed to the last known address of all the families whose name had been obtained from the Régie des Rentes du Québec. A problem was posed for us as the Family Allowance lists do not contain the telephone numbers. Thus, we were obliged to look up the telephone numbers of all the families in the street directory as well as the local telephone directory.

This introductory letter explained the study, its design and purpose, and informed them that they would be contacted soon by telephone by someone from our group to discuss participation in the study. A few days after this mailing, 10 telephone interviewers were assigned to contact the households, establish eligibility and amongst the eligible households attempt to elicit participation. The eligibility rates were much lower than expected (Table 3). A significant proportion of contacts reported consumption of bottled or filtered water as the main source of water). In addition, 225 families included in the original sampling frame of 3200, actually lived outside the area served by the treatment plant. We therefore asked the Family Allowance Board to send us the remaining 1600 names on their list. It was decided to complete as far as possible recruitment to the first three groups and use the remaining names as the sampling frame for the fourth group. Remaining names at the end of the process were randomly assigned to compensate for losses occurring within the groups. Because losses were higher in the group assigned bottled tap water, a larger proportion of families were assigned to this group. If a family agreed to participate during the telephone conversation, it was randomly assigned to one of the groups and an interviewer was dispatched to the house. The purpose of this visit was to carefully explain what we wanted the family to do with the Health Calendar and the water consumption questionnaire. In addition, a questionnaire on sociodemographic factors, chronic medical conditions, medication used and regular water consumption was delivered. The enrollment process was completed at the end of December 1993. The first three groups were recruited and commenced participation between the 13th of September and the end of October 1993 (Figure 3). We received the sampling frame for the fourth group in November 1993. Recruitment of all groups and the installation of the purge valves was completed by the end of December 1993. The population was certainly showing signs of distress and unwillingness to undertake any activity no matter how useful to society. This was exacerbated by the fact that the two bottled water groups were being asked to agree to exert considerable effort to participate. Thus, we saw a higher initial refusal rates in the two bottled water groups than in the tap water groups. In addition, compliance after initiation of the study was a problem in these two groups necessitating an oversampling strategy to maintain a sufficiently large sample size.

Table 3 Response to request to participate in the study in each of the four study groups

	RO water		Tap water		Plant water		Tap-valve	
	n	%	n	%	n	%	n	%
Total number of families randomized to the group	1206	100	883	100	1203	100	1050	100
Unable to reach	86	7.1	11	1.2	57	4.7	10	1.0
Not eligible - drinks modified water	217	18.0	213	24.1	226	18.8	259	24.7
Not eligible - no child	15	1.2	12	1.4	5	0.4	6	0.6
Not eligible - not able to communicate in French	8	0.7	9	1.0	13	1.1	11	1.0
Refused when first called	257	21.3	141	16	345	28.7	182	17.3
Agreed to participate when first called	387	32.1	373	42.2	419	34.8	369	35.1
Remained committed when visited	371	30.8	360	40.8	405	33.7	355	33.8
Participating (Dec. 1993)	339	28.1	346	39.2	354	29.4	330	31.4
Participating (Dec. 1994)	270	22.3	296	33.5	215	17.9	281	26.8

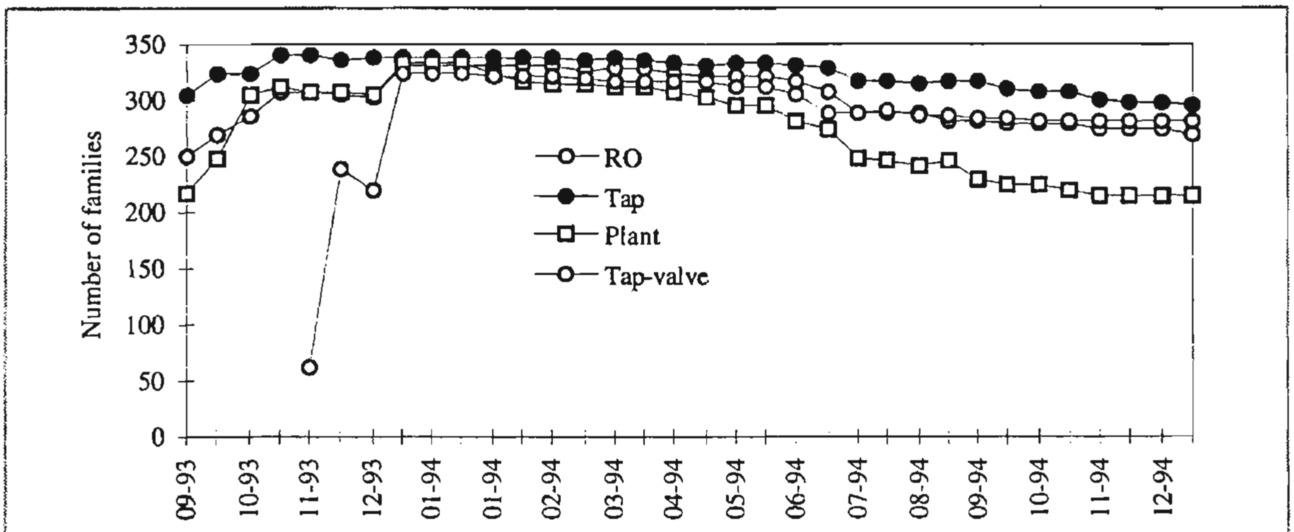


Figure 3 Enrollment and participation in the four study groups.

Enrollment of the subjects was attained within 2 months for the first 3 groups. The fourth group was a later addition to the design and enrollment proceeded from period 5 until the Holiday Season 1993. All subjects had been enrolled by the end of 1993 (Figure 3). The number of participating families remained above 270 for all groups except for the Plant group in which it decreased

gradually to 215 families. Period 21, end of June, shows a loss of participating families. July 1st, in Quebec is the official day when people renew their lease for their apartments. Houses are sold and bought using that date as the preferred date and a large number of families move to their new apartment or house. If the family moved within the studied area, they were asked to continue participation in the same group. For subjects from the Tap-valve group, we installed a similar valve in their new household.

Some subjects in the Plant water group complained of the taste of the water. Bottled plant water had a distinctive taste and odor that a few subjects found obnoxious enough to terminate their participation in the study. While we were able to keep most subjects, the rate of attrition in this group was higher.

3.2 SOCIODEMOGRAPHIC DATA

Subjects were equally randomized in the four study groups by age, sex, region, ethnic origin of the family and family income (Table 4). Differences were observed in region 2 and 5, but this is not outside a normal randomization especially with this variable.

Table 4 Socio-demographic distribution of subjects in the four observation groups.

		GROUP								
Variable	Sub-variable	RO		Tap		Plant		Tap-valve		Total
Age	0-5	206	26%	166	21%	200	25%	214	27%	786
	6-20	499	26%	490	25%	476	25%	458	24%	1923
	21-49	618	25%	616	25%	591	24%	601	25%	2426
	50+	37	31%	24	20%	30	25%	27	23%	118
Sex	Female	706	26%	678	25%	668	25%	656	24%	2708
	Male	654	26%	618	24%	629	25%	644	25%	2545
Region	#1	303	24%	264	21%	367	29%	340	27%	1274
	#2*	150	18%	253	31%	215	26%	199	24%	817
	#3	273	24%	304	26%	296	26%	282	24%	1155
	#4	186	21%	253	28%	223	25%	228	26%	890
	#5	448	40%	222	20%	196	18%	251	22%	1117
Income	<$20K	159	29%	104	19%	190	35%	96	17%	549
	$20-40K	375	26%	347	24%	309	22%	402	28%	1433
	$40-60K	348	28%	286	23%	214	17%	376	31%	1224
	$60K+	275	23%	364	30%	273	22%	307	25%	1219
	Unknown	203	25%	195	24%	311	38%	119	14%	828
Ethnic origin	Quebec	1042	26%	970	24%	973	25%	980	25%	3965
	Other	299	26%	275	24%	289	25%	289	25%	1152
	North American	15	14%	34	33%	31	30%	24	23%	104
	Unknown	4	13%	17	53%	4	13%	7	22%	32
	Total	1360		1296		1297		1300		5253

* *Plant is located in area 2*

Table 5. Average results recorded at the water treatment plant.

	Average	Maximum	Minimum
Water temperature (°C)	10.9	22.7	0.0
pH, raw water	7.4	7.6	7.2
pH, treated water	7.7	8.0	7.7
Residual chlorine (mg/L)	0.4	0.6	0.4
Maximum production (m3/d)	70 482	82 000	53 000
Filtration rate, plant #1 (m3/d)	30 374	44 500	20 200
Filtration rate, plant #2 (m3/d)	44 015	50 500	32000
Residual ozone (mg/l), plant #1	0.3	0.4	0.3
Residual ozone (mg/l), plant #2	0.3	0.4	0.3

Turbidity (NTU)

Settled water Plant #1	0.54	1.01	0.33
Settled water Plant #2	0.61	0.95	0.36
Filter (SA) no. 1 Plant #1	0.06	0.07	0.04
Filter (SA) no. 2 Plant #1	0.03	0.05	0.02
Filter (SA) no. 3 Plant #1	0.12	0.18	0.08
Filter (SA) no. 4 Plant #1	0.04	0.06	0.02
Filter (SA) no. 5 Plant #1	0.05	0.08	0.04
Filter (SA) no. 6 Plant #1	0.03	0.03	0.02
Filter (SA) no. 7 Plant #1	0.15	0.23	0.12
Filter (SA) no. 8 Plant #1	0.03	0.05	0.01
Filter (SA) no. 9 Plant #1	0.05	0.07	0.02
Filter (SA) no. 10 Plant #2	0.08	0.10	0.07
Filter (SA) no. 11 Plant #2	0.06	0.07	0.04
Filter (SA) no. 12 Plant #2	0.06	0.07	0.04
Filter (SA) no. 13 Plant #2	0.12	0.23	0.03
Filter (CS) no. 14 Plant #2	0.10	0.28	0.06
Filter (CS) no. 15 Plant #2	0.17	0.26	0.11

SA = Sand-anthracite filter
CS = GAC -Sand filter

3.3 WATER QUALITY

3.3.1 Treatment plant results

3.3.1.1 Physico-chemical data

The plant produced water that met or exceeded current North American water regulations. Turbidity was maintained to less than 0.1 NTU after filtration (Table 5). A detectable level of chlorine residual of at least 0.4 mg/L was maintained at all time in the distribution system. Temperature varied from 0°C to 24°C.

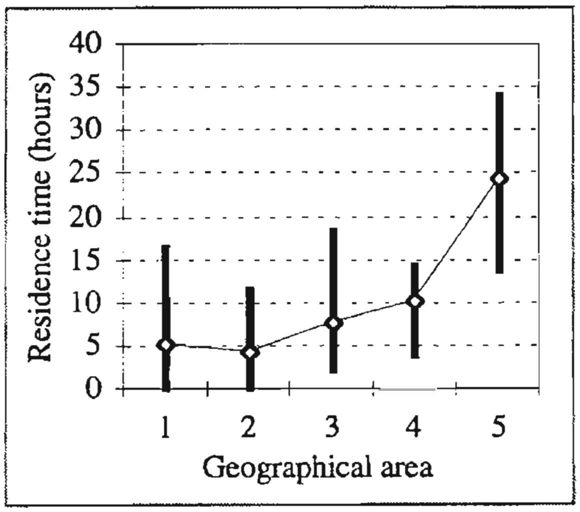
Winter of 1993-94 was one of the coldest experienced in Quebec for decades. From early November 1993, temperatures were extremely low and often below to -20°C. This cold spell lasted until April 1994 and resulted in water becoming colder earlier and staying cold for longer than usual. Problems with frozen service lines were frequent during that period, but no violations of the coliform rule were recorded.

3.3.1.2 Residence time in distribution system

Average residence time of water for each household was calculated using a computer model of the distribution system (Table 6).

Table 6 Estimated residence time for each geographic area.

Area	Average	Maximum	Minimum
1	5.1	16.5	0.3
2	4.4	11.5	0.3
3	7.7	18.3	2.3
4	10.1	14.2	4.2
5	24.5	34.0	14.0



For areas 1, 2, 3 and 4, which are geographically closer to the treatment plant, the average residence time are relatively similar. Area 5, has an average residence time of 24.5 hours with a minimum of 14 hours and a maximum of 34 hours, correlating with the increased distance from the plant.

3.3.1.3 Microbiological data and compliance data

3.3.1.3.1 Microbiological data from water treatment large volume analyses

Microbiological analysis of river water entering the water treatment plant revealed high levels of human enteric viruses and parasites, especially *Giardia* cysts (Table 7). The two indicators measured, *Clostridium perfringens* and somatic coliphages were also found in large numbers. Some were found in filtered water(Table 7, Figure 4, Figure 5) and treated water was free of these microorganisms.

Human enteric viruses were present in relatively high numbers (410 mpniu/100L) in the samples of the river water. None were detected in any of the filtered or finished water samples. The detection limit was 0.3 mpniu/100L. The number of viruses detected was relatively stable until April 1994, when their numbers sharply dropped after the spring run-off. Virus numbers then increased until they were at original levels by the end of the study in December 1994.

Somatic coliphages were detected in large numbers (geometric mean: 27,000 pfu/100L) in all samples of raw water and most filtered water samples. None were found in the finished water samples at a detection limit of 2 pfu/100L.

Clostridium perfringens were present in all raw water samples (geometric mean: 233,000 cfu/100L) and were detected in 30% of filtered water samples. None were found in the finished water samples (0/32) at a detection limit of 20 cfu/100L. These bacteria were the most numerous in river water and their numbers were relatively stable over the period of the study.

Giardia cysts were present in all raw samples and in one filtered water sample at the detection level. None were found in the finished water samples at a detection limit of 0.1 cyst/100L.

Cryptosporidium oocysts were found in 48% of the raw water samples and in three filtered water samples (close to detection limit). After treatment, a few unconfirmed structures were observed in very low numbers, but we had no means of confirming what they were. Because they were not classical oocysts structure they have not been considered as oocysts. The detection limit was 0.1 oocyst/100L.

Table 7 Microbiological results (geometric mean) from analysis of weekly water samples collected at the water treatment plant.

Microorganism	Raw water g.m.	Raw water P/N	Filtered water g.m.	Filtered water P/N	Filtered water Removal	Finished water g.m.	Finished water P/N	Finished water Removal
Clostridium perfringens (cfu/100L)	233,000	33/33	30	9/33	3.8	<10	0/32	5.0
Somatic coliphages (pfu/100L)	27,000	33/33	15	24/32	3.2	<1	0/32	5.0
Enteric viruses (mpniu/100L)	410	31/33	<0.3	0/32	4.1	<0.3	0/32	4.1
Giardia (cysts/100L)	200	33/33	0.2	1/32	3.1	<0.1	0/32	4.3
Cryptosporidium (oocysts/100L)	14	15/33	0.3	7/32	1.7	<0.1	0/32	3.2

g.m. = geometric mean
P/N = positive samples / number of samples
Removal = mean observed removal in log10. This value was computed using the actual number of microorganisms in raw water as the basis. For filtered or finished water negative samples, the assumed value was set at the detection limit.

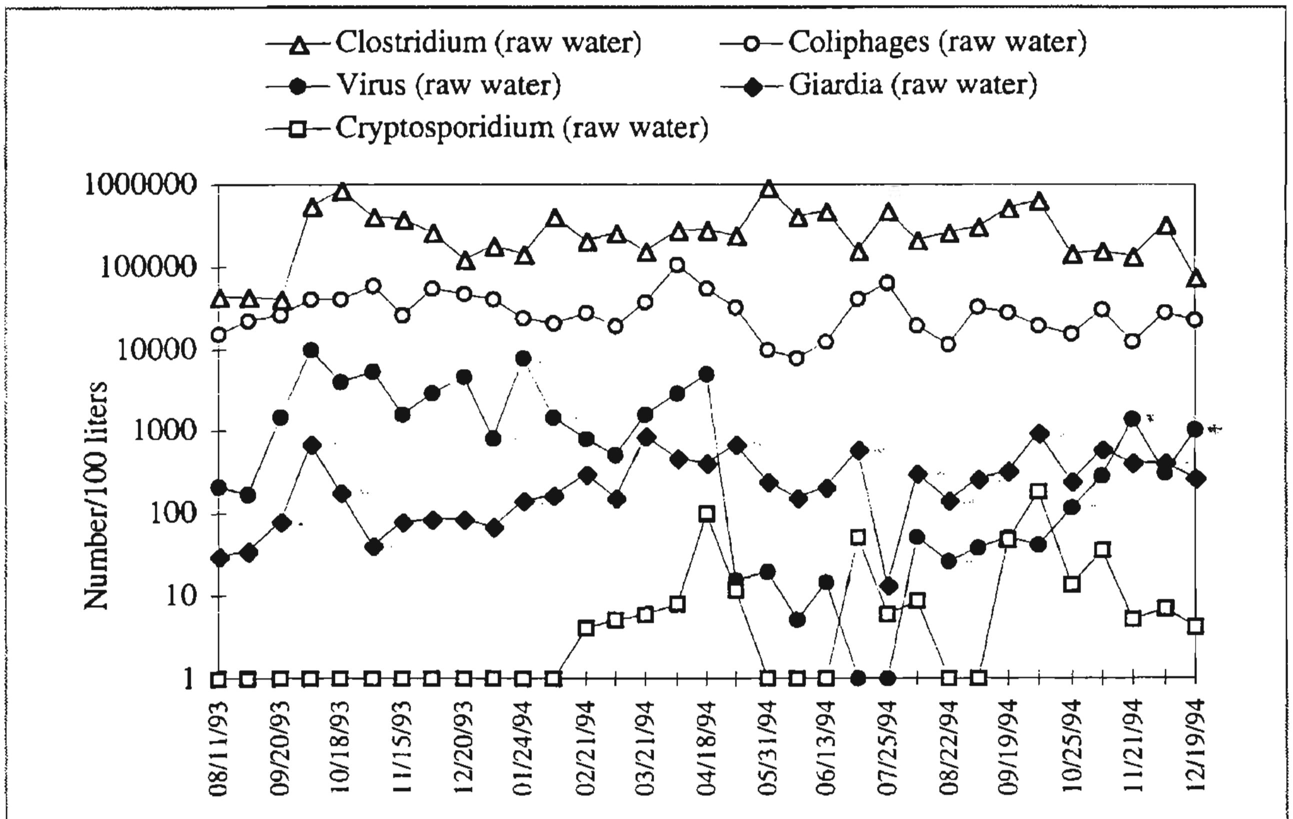


Figure 4 Microorganisms in river water entering the water treatment plant.

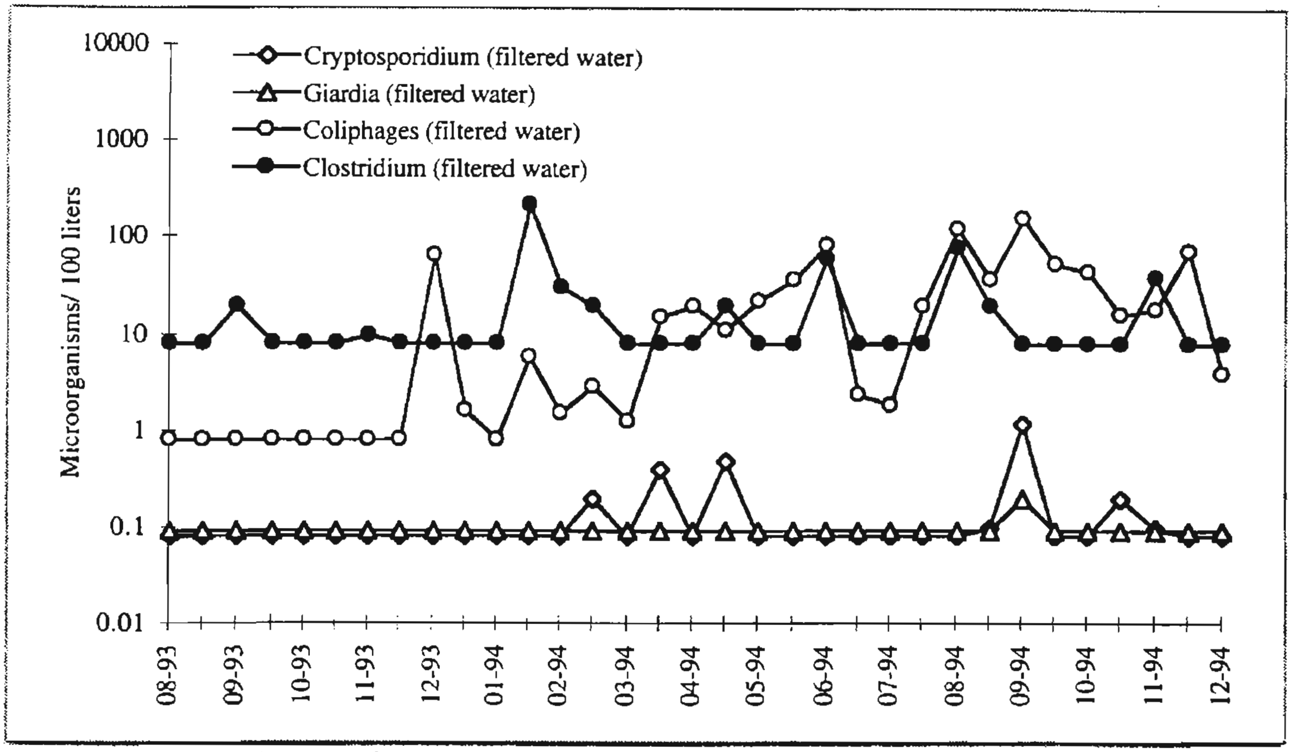


Figure 5 *Clostridium perfringens*, somatic coliphages, *Giardia* cysts and *Cryptosporidium* oocysts in filtered water before application of any disinfectant.

The flat section of each line indicates the detection limit for each microorganism.

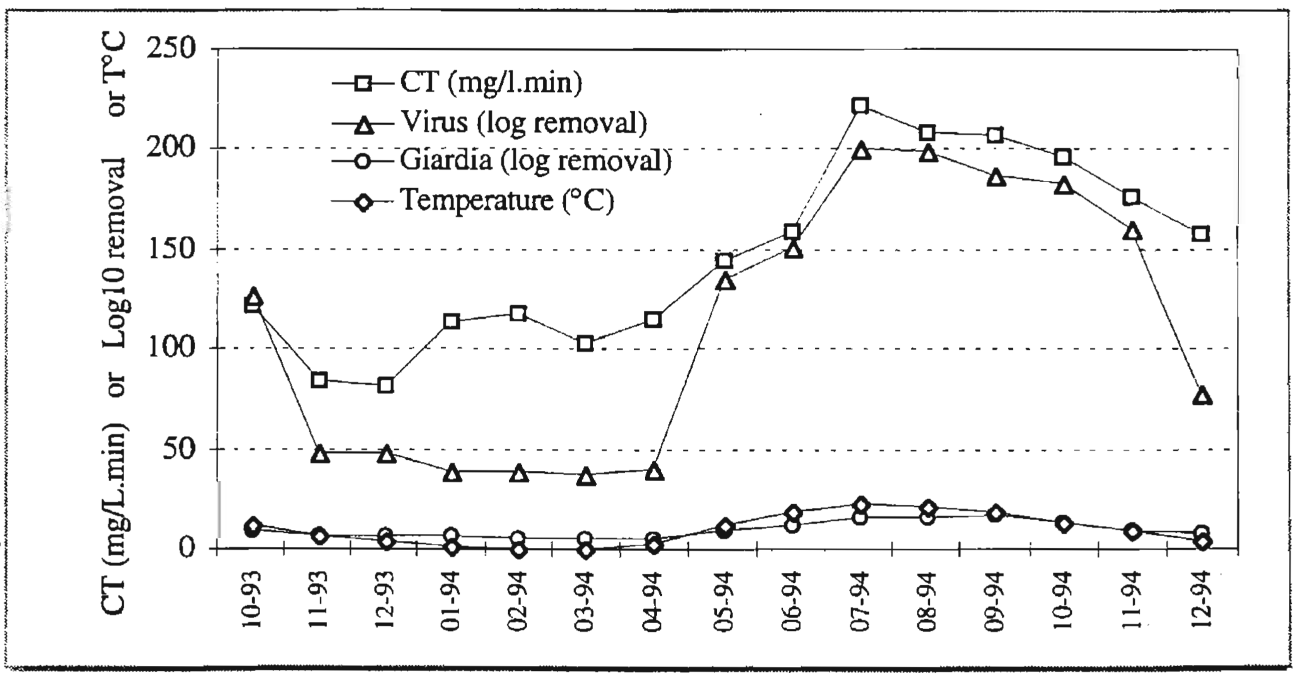


Figure 6 Temperature, measured Ct values and predicted inactivation and removal of viruses and *Giardia* at the water treatment plant.

3.3.1.3.2 Compliance monitoring data

Over the period of the study, the bacteriological quality of the treated water was always within current North American guidelines. Compliance rate was excellent with 0.6% of the samples failing because of total coliforms and none with fecal coliform.

Ct values computed from the plant data and tracer experiments are presented in Figure 6. Giardia and virus inactivation at each step of treatment are presented in Figure 8 and Figure 9.

After filtration, treatments had physically removed at least 2 logs of *Giardia* cysts and 4 and 6 log of *Clostridium perfringens* before any disinfection by ozone and chlorine. Estimated minimum log10 removal were calculated using values in raw water compared to values in filtered water. Data are presented in Figure 7

Performance of the plant was evaluated by a private consulting firm whose mandate was to estimate the Ct (disinfectant concentration x time) values achieved under operational conditions using the published USEPA methods (Federal Register 1989), tracer experiments and data from the plant. The models predicted Ct10 values of 82 to 221 mg/l.min or an extrapolated to 5 to 17 log of *Giardia* and 45 to 200 logs of viruses (Figure 6, Figure 8, Figure 9).

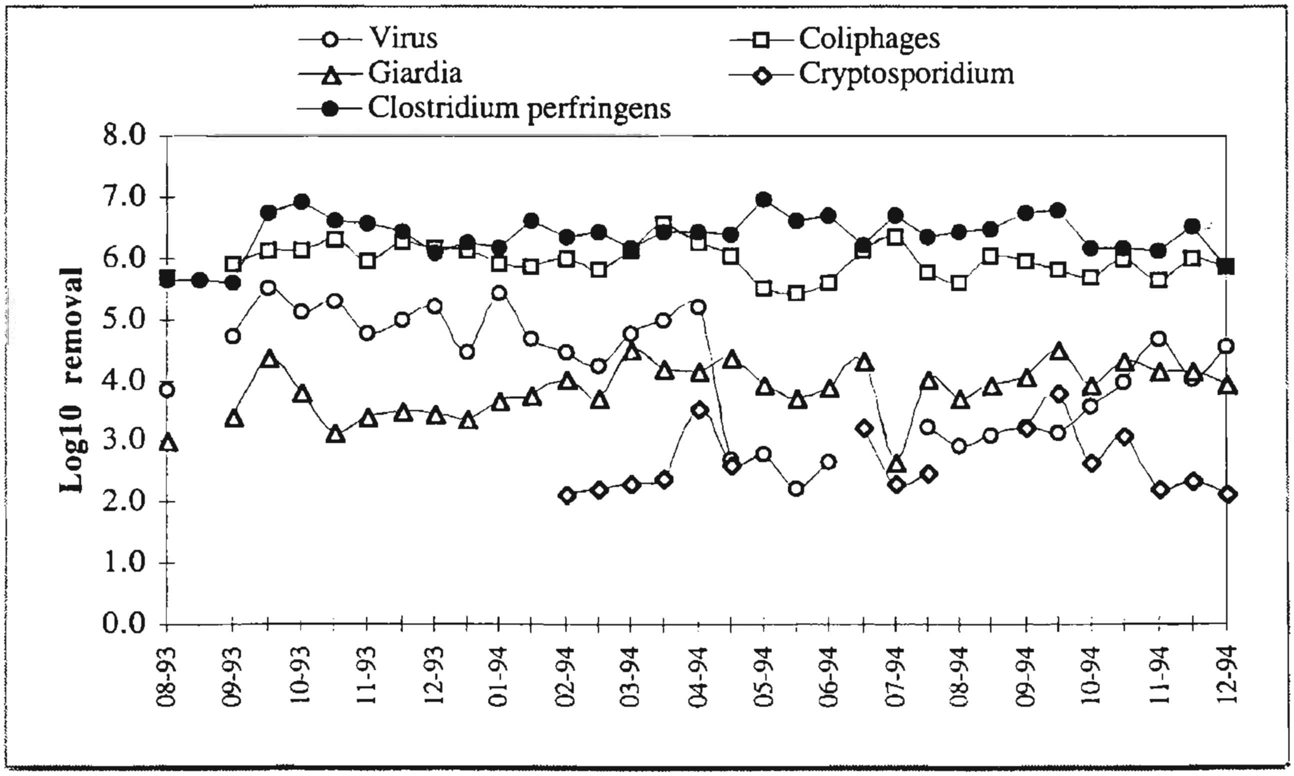


Figure 7 Estimated **minimum observed** log10 removal after filtration.

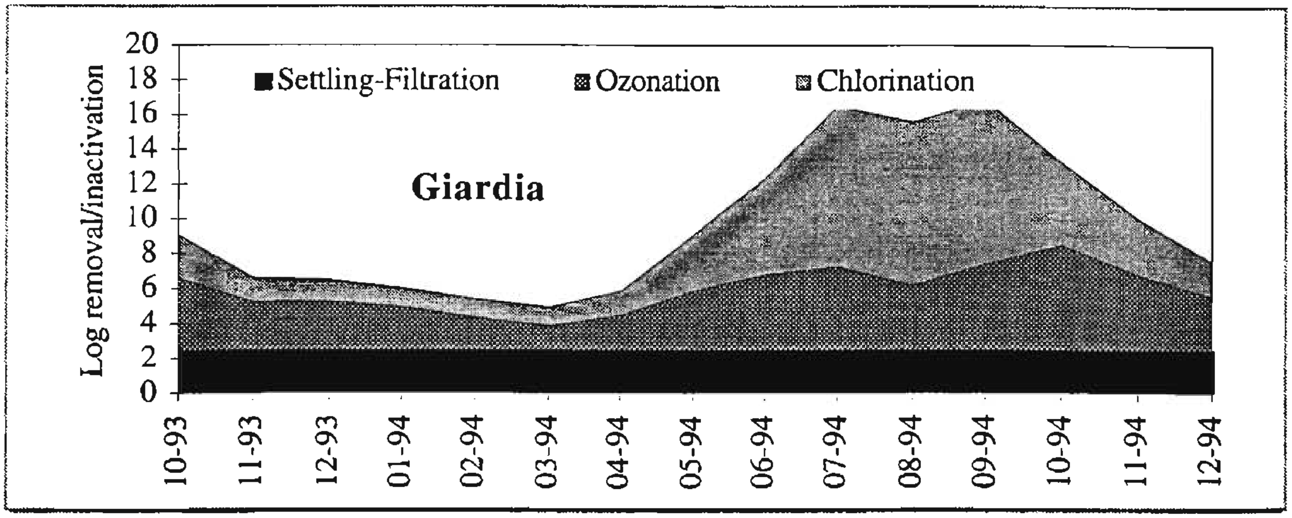


Figure 8 Measured CT and estimated log removal and inactivation of Giardia cysts at each step of treatment.

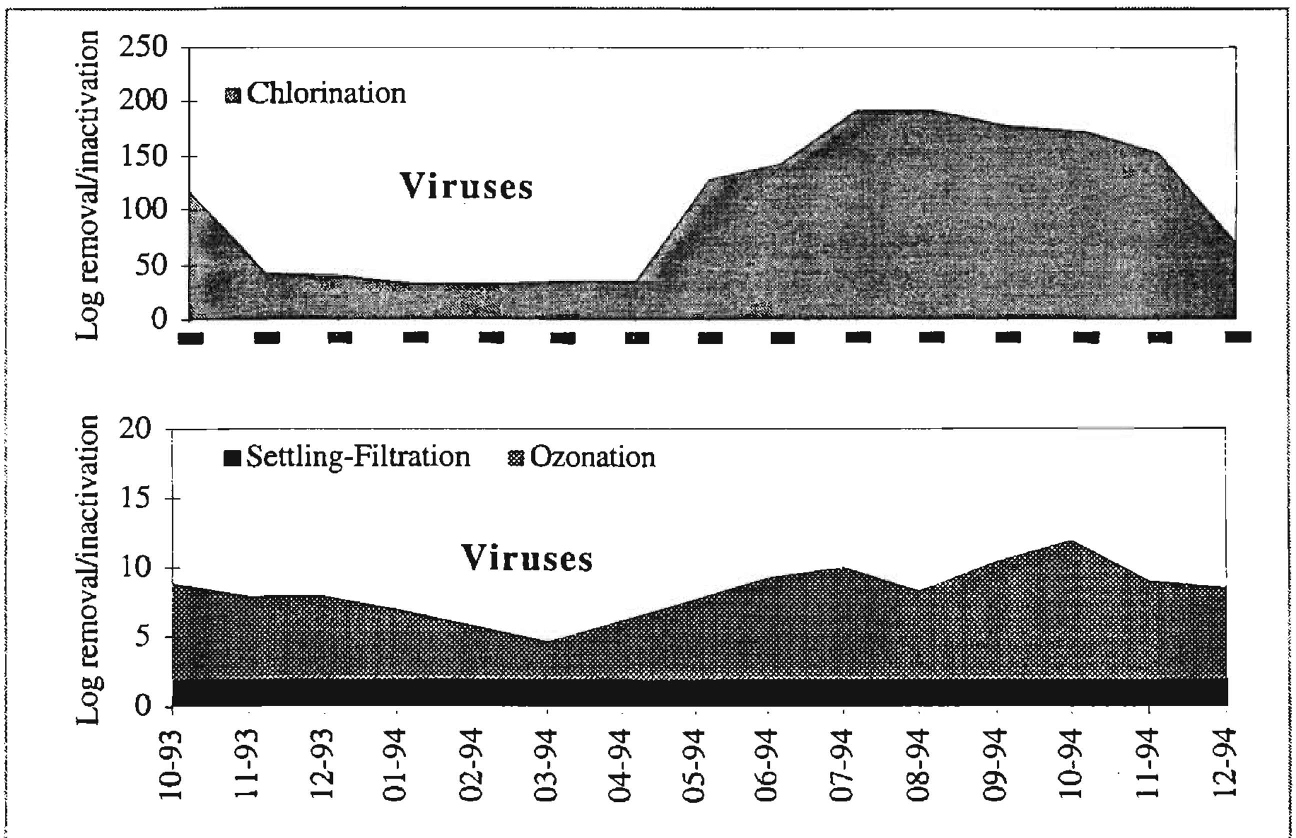


Figure 9 Estimated log10 removal and inactivation of human enteric viruses at each step of treatment.

Table 8. Comparison of bacterial contamination of tap water at households with a bleeder valve, at the nearest neighbor and at the nearest main connection.

	Flush time	HPC 35°C			HPC 25°C			Total viable counts (Epifluorescence)		
Site	(min.)	Neighbor	Valve	Main	Neighbor	Valve	Main	Neighbor	Valved	Nearest
#1	0	95	3,650	40	1,000	51,300	30	362,895	572,030	572,030
	1	1	6	nd	315	3,070	nd	101,278	63,577	63,577
	2	0	3	nd	275	1,145	nd	100,212	nd	nd
	3	0	2	nd	190	790	nd	89,906	nd	nd
	4	0	3	nd	225	410	nd	57,995	nd	nd
	5	0	10	1	220	nd	120	64,391	54,273	54,273
	7	0	7	nd	150	330	nd	71,912	nd	nd
	10	0	5	nd	170	355	nd	47,517	74,981	74,981
	25	1	0	2	160	85	40	42,075	50,397	50,397
#2	0	615	4,000	100	2,000	13,500	400	89,338	68,229	68,229
	1	110	nd	nd	935	nd	nd	nd	nd	nd
	1	61	nd	nd	935	nd	nd	51,172	nd	nd
	2	90	nd	nd	835	nd	nd		nd	nd
	5	50	47	78	405	2,885	685	48,613	nd	nd
	10	45	44	98	280	1,920	235	64,818	nd	nd
	15	53	nd	nd	175	nd	nd	nd	nd	nd
	20	49	50	45	210	2,265	225	52,025	46,908	46,908
#3	0	910	nd	20	2,930	nd	275	162,045	nd	nd
	1	68	nd	nd	725	nd	nd	93,815	nd	nd
	1	66	nd	nd	185	nd	nd	43,496	nd	nd
	2	67	nd	nd	875	nd	nd	46,183	nd	nd
	5	69	nd	23	335	nd	180	44,434	nd	nd
	10	70	nd	28	430	nd	218	nd	nd	nd
	15	62	nd	24	570	nd	161	nd	nd	nd
	20	72	nd	31	510	nd	119	34,115	0	nd

3.3.2 Households with purge valves

Bacteriological analyses were performed at three pilot sites where purge-valves were installed at the onset of the study. These results indicate that the installation of a purge valve brought water to the same bacteriological quality observed in the mains.

This was also confirmed by the bacteriological analysis of water samples obtained during visits to households with valves (Table 9). HPC counts were lower than would have been expected for normal taps.

Table 9. Bacteriological analyses of water samples collected during visits to households with valves installed.

	HPC 35		HPC 25	
Flush period	GeoMean	n	GeoMean	n
0 min.	44	29	288	31
1 min.	3	35	71	32
2 min.	5	114	77	107

Results obtained after letting the water run at full flow for the indicated time. All values are expressed as cfu/100 ml.

3.3.3 Bottled water

3.3.3.1 Plant water

On several occasions bottled tap water from the treatment plant had an aggressive metallic or chlorinated taste. Some subjects refused to drink this water and several families dropped-out of the study. It appears that this was limited to a small proportion of the population that had the ability to taste or smell some treatment by-products. We have not been able to identify the source of these problems.

Bottled water from the water treatment still contained enough nutrients to support bacterial growth. Pilot experiments showed that the bacterial counts progressed rapidly and after 10 to 14 days could reach to 200,000,000 cfu/100 ml (HPC 25°C).

Bacteriological analysis of plant water revealed very little contamination at the time of bottling (Table 10). These analysis were performed on every lot of bottled water. Results 3 to 5 days after bottling revealed HPC counts at 25°C which were less than 10 cfu/100 ml and less than 1 cfu/100 ml at 35°C. The second set of analyses, 2 weeks later, revealed higher bacterial counts at 25°C and 35°C at respectively 1,273,700 and 4,997 cfu/100 ml. *Pseudomonas aeruginosa* or *Aeromonas hydrophila* were rarely detected. Some residual chlorine was also detectable after 2 to 4 days.

3.3.3.2 Bottled water (spring and RO treated)

Upon start-up, the RO-unit produced water that contained elevated bacterial counts. Operation of the RO-unit for a few minutes and discharging the water to the drain rapidly decreased bacterial counts to less than 100 cfu/ml (Figure 10). Spring and RO-filtered water were ozonated at the time of bottling, an operation that further decreased bacterial counts.

No aesthetic complaints were reported by consumers of these waters. Very little bacterial regrowth was observed (Table 10). Spring water was also ozonated at the time of bottling. When this water was delivered to our facilities, it had no significant bacterial content. There was no aesthetic complains by consumers of spring water. The results of bacteriological analyses (2 weeks later) (Table 10) generally indicated low bacterial counts with some bottle to bottle variation.

Table 10. Results of bacteriological analysis of bottled RO-treated, spring and plant water after 2 weeks at room temperature.

	Total counts (epifluorescence)	HPC 25°C	HPC 35°C	*Pseudomonas aeruginosa*	*Aeromonas hydrophila*	Total Coliforms
RO-treated water	3,680,200	0	0	0	0	0
Spring water	1,106,500	8	1	0	0	0
Plant water	63,714,300	1,273,700	4,997	0	0	0

Geometric count of number of bacteria per 100 ml (total counts) and cfu per 100 ml others.

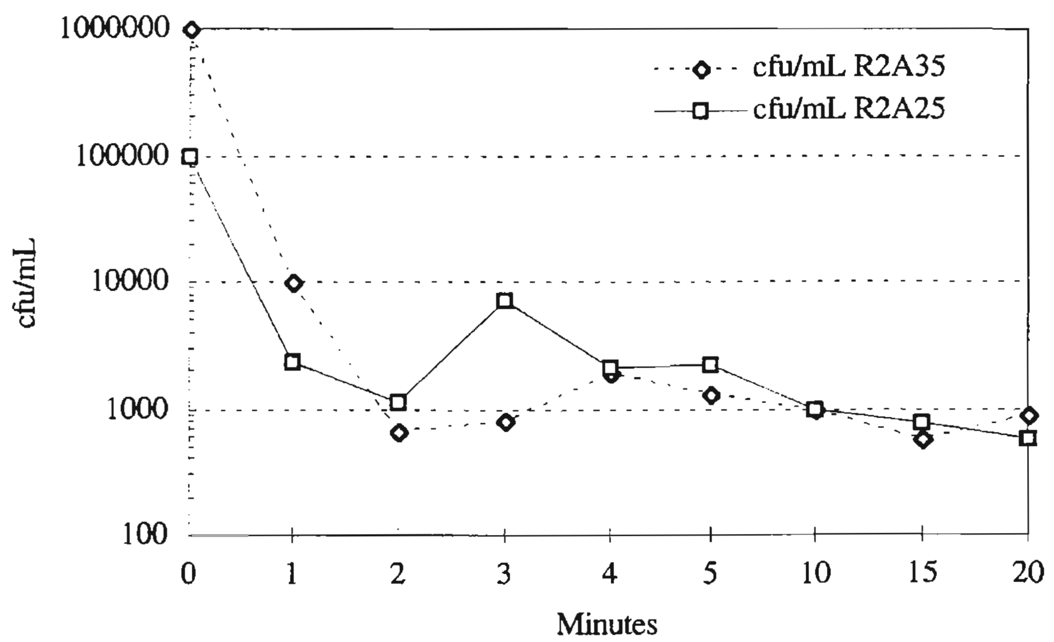


Figure 10 Effect of flushing on bacterial counts in water produced by reverse-osmosis unit installed at the water treatment plant.

Table 11. Type of water consumed by subjects in the four study groups.

Date	RO	Plant	Tap	Tap-valve
12-Sep-93	Spring water (bottled)	Plant water (bottled)	Tap water	
26-Sep-93	Spring water (bottled)	Plant water (bottled)	Tap water	
10-Oct-93	Spring water (bottled)	Plant water (bottled)	Tap water	
24-Oct-93	Spring water (bottled)	Plant water (bottled)	Tap water	
7-Nov-93	Spring water (bottled)	Spring water (bottled)	Tap water	Tap-valve water
21-Nov-93	Spring water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
5-Dec-93	Spring water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
19-Dec-93	??????	??????	Tap water	Tap-valve water
2-Jan-94	Spring water (bottled)	Spring water (bottled)	Tap water	Tap-valve water
16-Jan-94	Spring water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
30-Jan-94	Spring water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
13-Feb-94	Spring water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
27-Feb-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
13-Mar-94	Spring water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
27-Mar-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
10-Apr-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
24-Apr-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
8-May-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
22-May-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
5-Jun-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
19-Jun-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
3-Jul-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
17-Jul-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
31-Jul-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
14-Aug-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
28-Aug-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
11-Sep-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
25-Sep-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
9-Oct-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
23-Oct-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
6-Nov-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
20-Nov-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
4-Dec-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water
18-Dec-94	RO water (bottled)	Plant water (bottled)	Tap water	Tap-valve water

Legend:
- Spring water (bottled)
- RO water (bottled)
- Plant water (bottled)
- Tap water
- Tap-valve water

3.4 WATER USED BY EACH GROUP

3.4.1 Installation and verification of purge valves

Under the sink purge valves were installed by professional plumbers from a local company. The plumber adjusted the valve to about 500 ml/min and told the subjects not to close this valve. Later in the study, we sent a letter to all subjects explaining the importance of this valve and that it should remain opened.

There were problems associated with installing these valves on older plumbing. In several households, the existing plumbing had to be modified to accommodate the valves.

Valves were installed early winter when water temperature in the distribution system was about 1°C: the first problem encountered was condensation of humid air on the pipes resulting in property damages. This was solved by installing insulation on all exposed pipes.

A second problem was noise encountered with some installations. Both subjects and neighbors complained and we reduced the flow to a level at which the noise level was acceptable.

All households with valves were visited twice (May 1994 and September 1994) by a member of our team to insure that the valve had been left opened and that water was running. Water samples were collected during these visits for bacteriological analysis. About 15% of the valves were found closed and were reopened at that time.

3.4.2 Amount of bottled water delivered

The amount of bottled water that was delivered to the subjects is a good indication of the amount of water used (Table 12).

An order was placed weekly to the bottler, alternatively for Plant water and for RO-treated water. The average weekly order to the bottling company was about to 1200 cases (4 bottles of 4 liters). For the whole period of the study, almost 80,000 cases of bottled water were delivered to the subjects in these two groups for a total volume of over 1,250,000 liters of water. From this value, the average amount of water utilized was estimated at about 14 liters per person per 2-week period, roughly an average of 1 liter per day. This is consistent with the data obtained from the water consumption questionnaires (see section 3.4.3).

Table 12. Bottled water delivered to the subjects and estimated personal use.

Group	Average number of individuals in group	Number of liters delivered per 2 weeks	Mean number of liters per person per 2 weeks
Bottled water	1369	18,720	13.7
Plant water	1366	18,624	13.6

Cases delivered: 66 weeks x 1200 cases = 79,200 cases
Volume of water bottled: 79,200 x 16L = 1,267,200 liters

3.4.3 Water consumption

The type of water consumed by each group is presented in Table 11.

As a temporary measure, because the RO unit was not operational until early 1994, the group that was supposed to receive RO-treated water, received instead bottled spring water until the unit was functional in March 1994. Even then, because of sub-zero temperatures (-20°C and lower), it was not possible to load the tanker as the treated water rapidly froze in the tanker reservoir. Spring water was used again as a replacement for a 2-week period. RO-treated water was then delivered on a regular schedule from April 1994.

During the Holiday season, subjects in the RO and Plant groups probably drank tap water even if some remaining bottled water might have been available. Subjects in the Tap and Tap-valve groups drank their tap water as usual throughout the observation period. We also did not collect information during that period. The Holiday period has been excluded from calculations of rates.

The water consumption questionnaires elicited data on drinking water obtained from bottled water, from the kitchen tap and the bathroom tap, and whether it was used for preparing hot or cold beverages. The data obtained from the kitchen and bathroom taps were combined for all analyses. The first questionnaire (Autumn 1993) and last questionnaire (Autumn 1994) presented some difficulties in interpretation. Some subjects returned these questionnaires with data obtained before they actually enrolled actively in the study or after they had terminated their participation in the study. It was relatively easy to exclude data from questionnaires with data after December 15 1994. It was more difficult to sort out the data from the first survey because subjects were not all enrolled at the same time. For analysis purposes, we have use the data from the June 1994 survey and compared it to the values obtained on subgroups known to be trustworthy in the two other surveys. Data from the three surveys shows that the amount of water consumed did not differ significantly in those three periods.

Total tap water consumption (Figure 11) for the two tap water groups was similar at 6.9 glasses per person-day in the Tap group and 7.0 glasses/person-day in the Tap-valve group with minimal bottled water consumption. Subjects in both bottled water groups had an equal consumption of bottled water at 4.2 glasses/day in the RO group and 4.3 glasses/day in the Plant group, but they also consumed about 3 glasses/day of tap water.

Figure 12 illustrates water consumption for unmodified tap or bottled water and where it is consumed. Less than one glass per day of water input is consumed out of the house and very little bottled water (probably including commercial water).

Figure 13 and Figure 14 illustrate the individual contribution of the subjects. As can be seen the maximum consumption of drinking water can be as high as 100 to 250 glasses per week (about 15 to 35 per day).

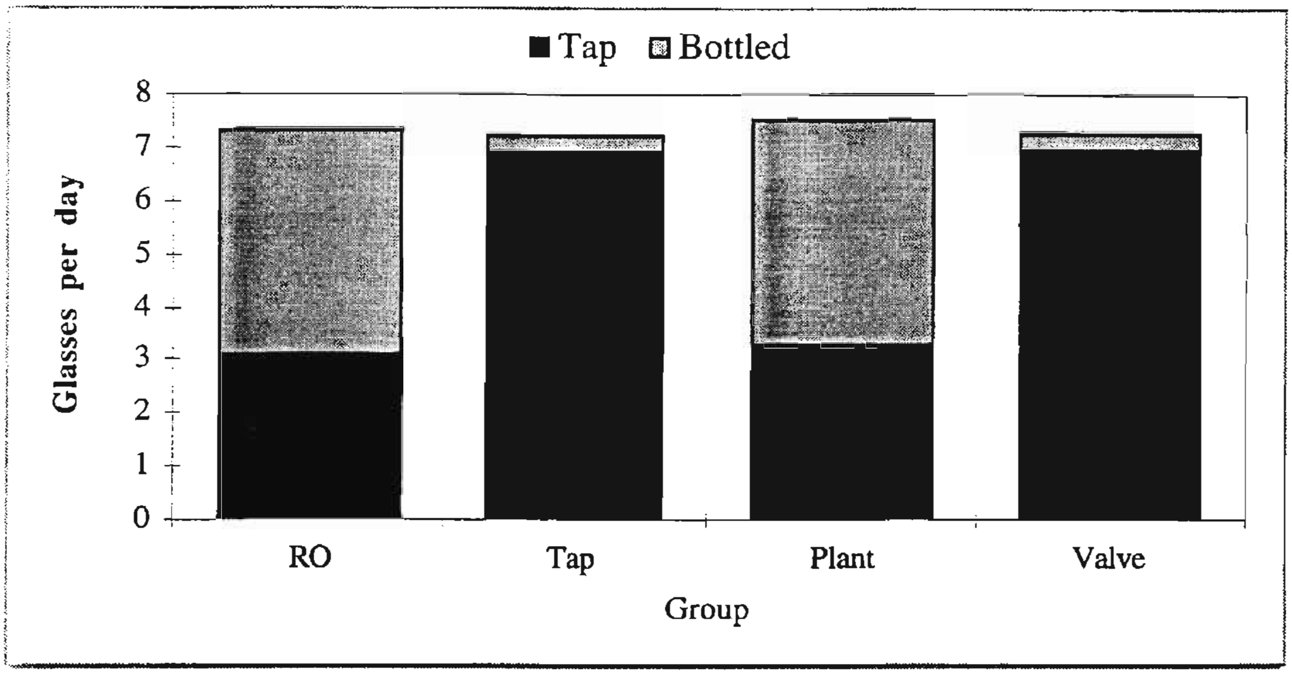


Figure 11 Total drinking water consumption (glasses per day per person) of bottled and tap water in the four study groups

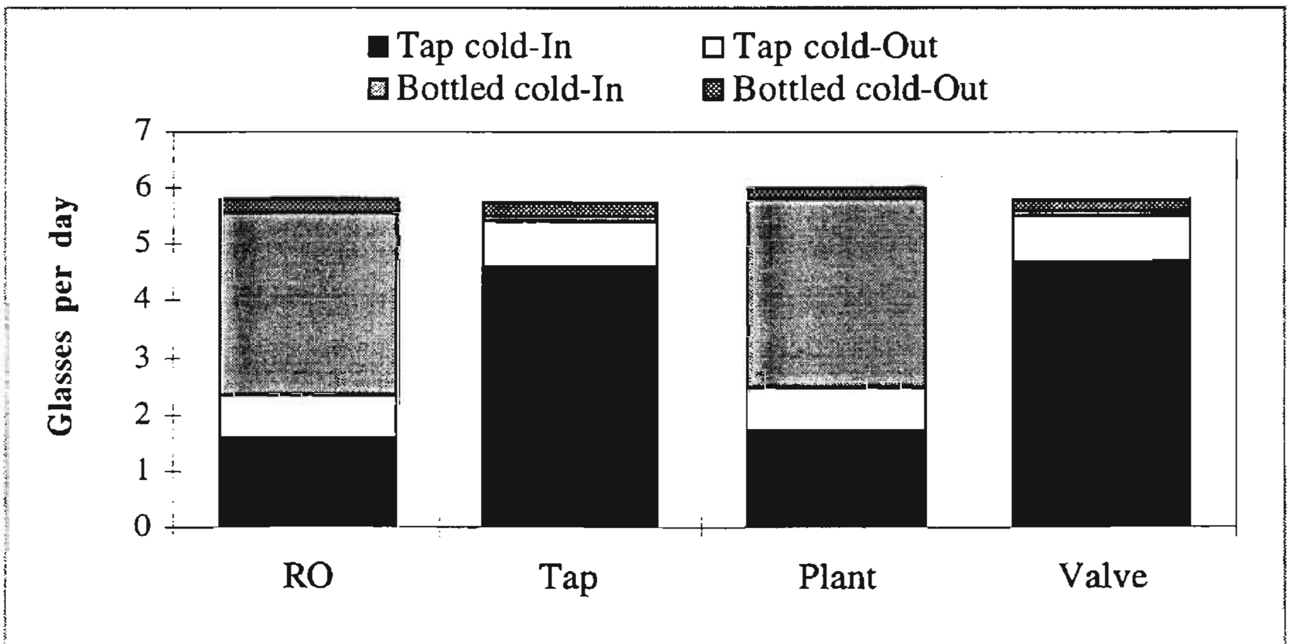


Figure 12 Unmodified drinking water (i.e., cold water) consumption by observation groups, location (in the house or outside) and type (bottled or tap).

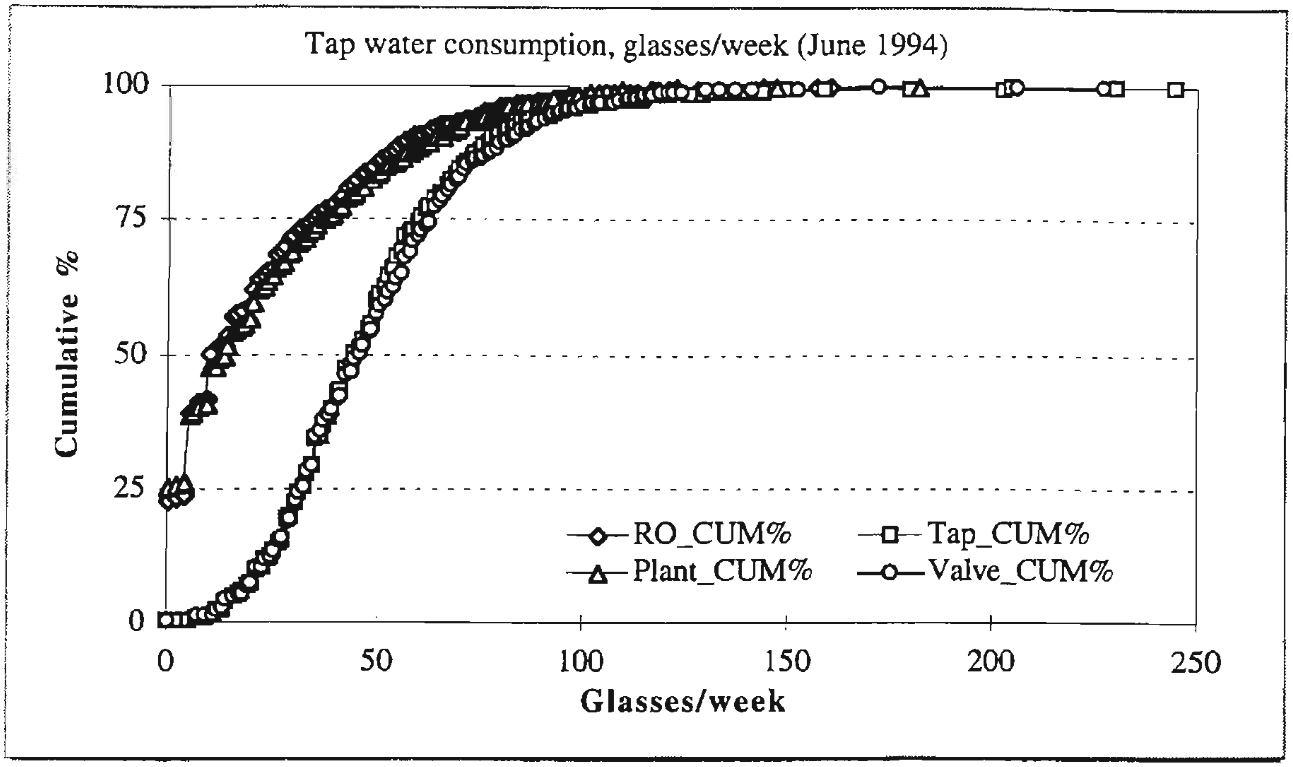


Figure 13 Cumulative frequency distribution of tap water (Tap or Tap-valve) consumption for subjects by observation groups.

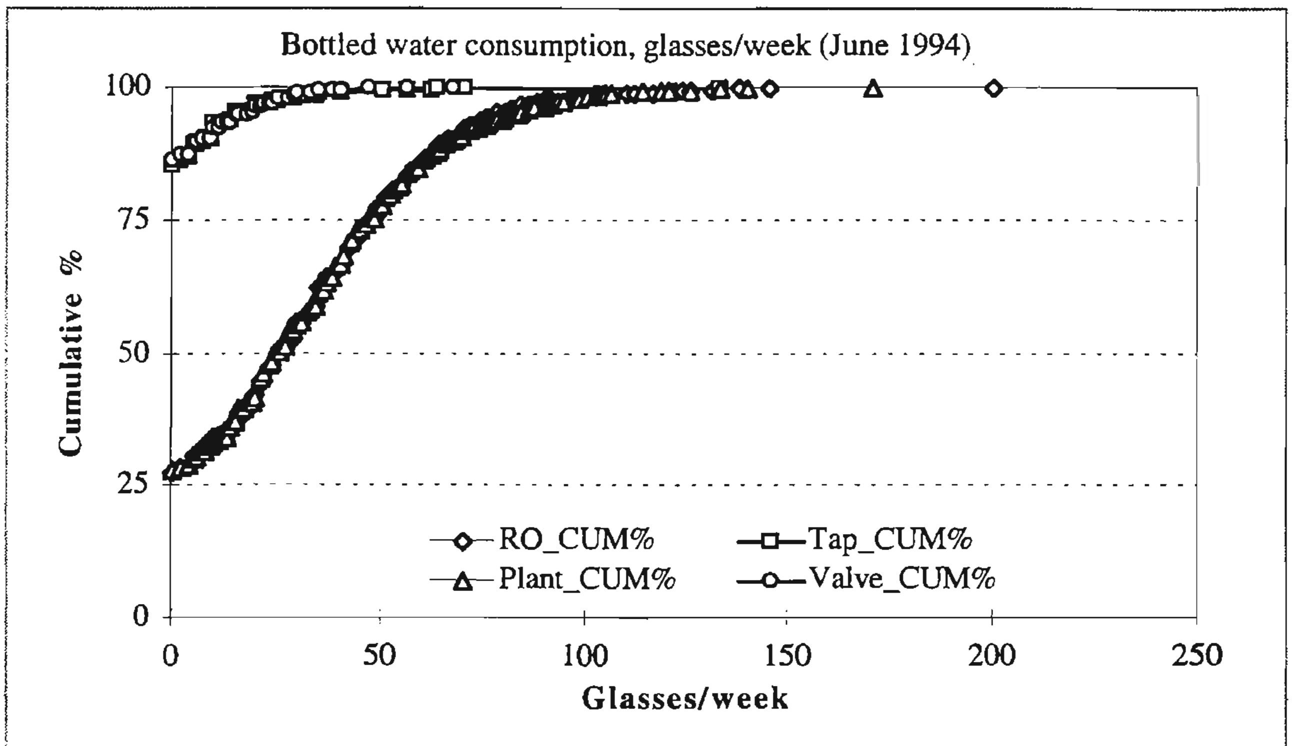


Figure 14 Cumulative frequency distribution of bottled water (RO or plant) consumption for subjects by observation groups.

3.4.4 Perception (taste) of the water

On each 2-week diary form, the respondent was asked to evaluate the taste of the water for the current period. A very large proportion of the subjects found the taste of the water to be good (Figure 15), about 25% of families in the RO water and 35% in the Tap-valve group found it excellent. In the Tap and Plant groups only 10% found it excellent.

It was also not surprising to find that 10% of the subjects in the Plant group found it bad tasting reflecting problems encountered with bottling plant water on several occasions.

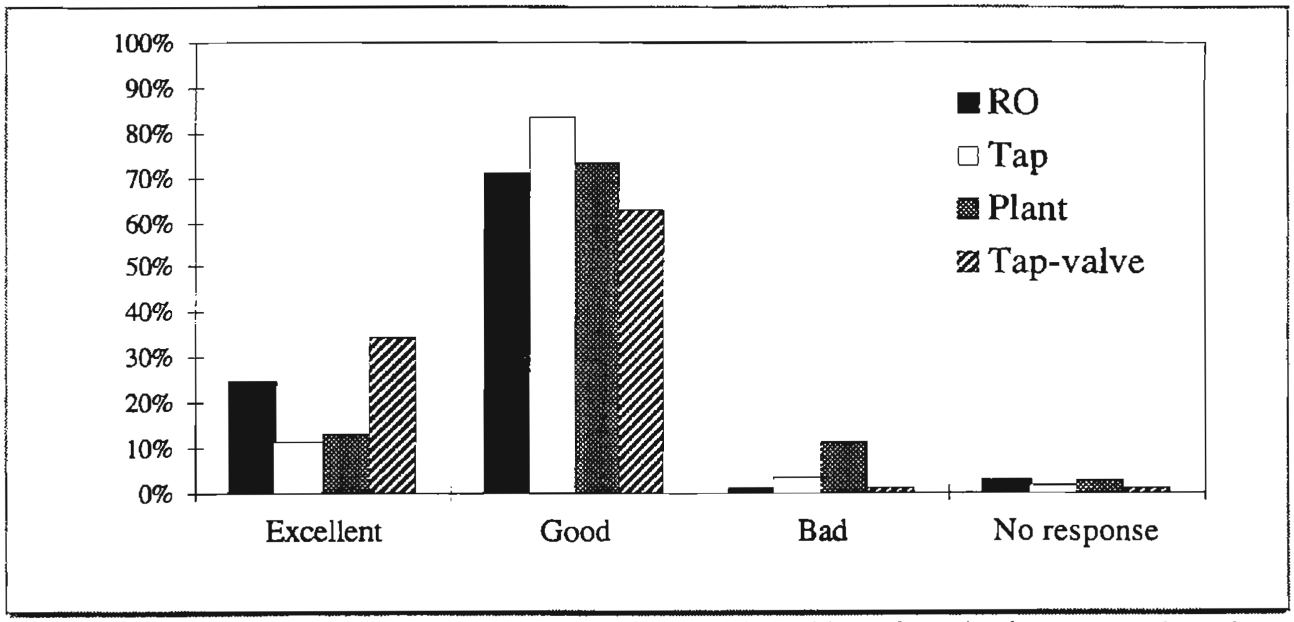


Figure 15 Evaluation of water quality (bottled and tap) by the subjects from the four groups based on 36000 responses from biweekly questionnaires.

3.5 RATE OF DISEASE IN THE POPULATION

3.5.1 Highly credible gastrointestinal illnesses (HCGI)

3.5.1.1 Crude data

It is important to note that subjects were first asked if they had experienced nausea, vomiting, diarrhea, Cold or Flu. All other symptoms are ancillary symptoms related to a positive answer to this question.

Individual symptoms were reported at variable rates and at higher rates for children (Table 13). The most frequently reported symptom was Cold and Flu in all groups. Subjects in the Tap and Tap-valve groups generally reported these symptoms more frequently while subjects in the Plant group reported these symptoms less frequently.

The level of water related events, other than seeking medical help is in excess for the subjects in the Tap and Tap-valve groups and is reduced for those in the Plant group (Table 13). The level of severity of these symptoms is suggested by the reports of changes in activities, absences, staying in bed, consulting a physician and being hospitalized. Hospitalization was a rare occurrence suggesting mild illnesses. The number of visits to physicians at a rate of 19 per 100 person-year in the RO and Tap group. This rate was increased by 24% for subjects in the Tap-valve group. A decrease of 24% was observed for the subjects in the Plant group: a similar decrease is observed for all events except Cold and Flu. The largest water related increases are observed for changes in daily activities and staying in bed with increase of 172% and 103% respectively for all subjects and similar values for children.

About 35% of the subjects experienced at least one HCGI episode during the observation period while in children this rate was closer to 40% (Table 14). These episodes lasted for 1 to 35 days, with the majority lasting 1 or 2 days (Table 15).

Table 13. Number of days the symptoms were reported per 100 persons-year for all subjects in the four observation groups.

Symptom	All subjects							2-12 years old						
	RO	Tap	Diff.	Plant	Diff.	Tap-valve	Diff.	RO	Tap	Diff.	Plant	Diff.	Tap-valve	Diff
Nausea	22.3	27.7	24%	20.2	-10%	32.7	46%	23.3	27.8	19%	15.6	-33%	40.2	72%
Vomiting	32.4	35.9	11%	30.9	-5%	43.0	33%	51.2	55.2	8%	46.9	-8%	62.5	22%
Diarrhea soft	51.7	69.6	35%	68.1	32%	60.7	17%	54.7	71.2	30%	59.4	8%	52.8	-4%
Diarrhea liquid	38.0	44.9	18%	52.4	38%	48.3	27%	41.7	49.2	18%	52.2	25%	44.1	6%
Diarrhea liquid, bloody	0.6	0.5		0.9		0.2		0.8	0.6		0.7		0.0	
Fever	44.9	47.7	6%	31.7	-29%	57.3	28%	68.2	79.1	16%	53.9	-21%	92.6	36%
Abdominal cramps	32.6	38.4	18%	31.7	-3%	41.1	26%	41.1	47.2	15%	36.1	-12%	50.5	23%
Muscular pains	7.7	16.4	113%	8.8	15%	12.9	68%	4.2	10.1	141%	4.9	18%	7.3	75%
Back pains	4.5	9.2	102%	5.0	9%	5.6	24%	2.0	3.5	75%	1.8	-9%	2.6	31%
Sore throat	36.1	57.1	58%	34.2	-5%	55.0	52%	42.5	60.4	42%	36.5	-14%	63.5	49%
Headache	31.0	57.1	84%	32.8	6%	53.2	71%	30.1	60.6	101%	28.6	-5%	58.0	93%
Cold or flu	132.1	152.1	15%	126.0	-5%	133.9	1%	166.8	195.1	17%	161.4	-3%	168.7	1%
Earache	9.2	10.1	10%	11.7	27%	10.4	14%	14.1	14.4	2%	14.7	4%	16.2	15%
Changed daily activities	20.8	39.2	89%	11.2	-46%	56.5	172%	27.2	53.6	97%	14.1	-48%	73.3	169%
Absent (work/school)	25.1	31.7	26%	17.6	-30%	36.8	46%	38.5	48.4	26%	28.6	-26%	55.4	44%
Stayed in bed	15.4	24.9	61%	9.4	-39%	31.2	103%	20.6	31.2	52%	11.9	-42%	39.6	93%
Consulted physician	19.0	19.4	2%	13.8	-28%	23.6	24%	28.0	29.3	5%	23.1	-18%	34.0	21%
Was hospitalized	0.7	0.5		0.4		0.6		0.9	0.5		0.0		0.6	

Rate Number of days the symptom was reported per 100 person-year

Diff. Percent difference with the RO group. Due to the large error introduced by observations occurring rarely, calculations for values less 1 day/100 persons-year are not presented.

Table 14. Frequency distribution of the number of HCGI episodes in the four observation groups for all subjects and for children 2-12 years.

	All individuals					Children 2 - 12 years old			
Number of episodes	RO	Tap	Plant	Tap valve*	Number of episodes	RO	Tap	Plant	Tap valve*
N =	1360	1296	1297	1300	N =	598	541	557	563
0	66.8%	62.4%	68.2%	63.9%	0	59.1%	50.9%	60.9%	56.1%
1	19.5%	20.8%	21.6%	22.5%	1	21.8%	25.2%	25.3%	25.0%
2	8.1%	9.6%	5.1%	7.2%	2	10.4%	13.0%	7.2%	11.1%
3	3.1%	3.7%	2.2%	3.5%	3	5.4%	5.7%	3.6%	4.5%
4	1.3%	1.6%	1.2%	1.2%	4	1.7%	3.0%	1.1%	1.2%
5	0.5%	0.8%	0.5%	0.8%	5	0.3%	1.1%	0.5%	1.1%
6	0.3%	0.5%	0.3%	0.5%	6	0.3%	0.7%	0.5%	0.7%
7	0.2%	0.2%	0.4%	0.1%	7	0.3%	0.0%	0.2%	0.2%
8 @ 16	0.3%	0.5%	0.5%	0.2%	8 to 14	0.7%	0.4%	0.7%	0.2%
At least 1	33.2%	37.6%	31.8%	36.1%	At least 1	40.9%	49.1%	39.1%	43.9%

Note: the observation period for the Tap-valve group is shorter by almost 2 months and the values presented for this group are thus underestimated.

Table 15. Frequency distribution of the duration of HCGI episodes in the four observation groups for all subjects and for children 2-12 years.

	All individuals					Children 2 - 12 years old			
Duration of HCGI in days	RO	Tap	Plant	Tap valve	Duration of HCGI in days	RO	Tap	Plant	Tap valve
N =	806	928	720	806	N =	445	472	366	427
1	59.3%	62.9%	54.7%	58.1%	1	60.0%	60.4%	59.3%	62.5%
2	17.8%	19.7%	20.5%	17.6%	2	18.7%	18.4%	19.7%	16.2%
3	8.6%	9.2%	7.9%	9.2%	3	7.4%	8.9%	5.7%	9.6%
4	5.9%	3.7%	4.0%	4.4%	4	6.1%	3.6%	4.1%	4.2%
5	2.6%	2.5%	2.9%	2.8%	5	2.7%	3.2%	2.7%	1.6%
6	1.3%	1.6%	2.3%	2.2%	6	1.1%	0.8%	2.2%	2.1%
7	1.5%	1.9%	1.6%	1.6%	7	1.3%	1.3%	1.9%	0.5%
8 @ 35	2.9%	3.6%	6.0%	4.0%	8 to 26	2.7%	0.8%	2.5%	0.5%

3.5.1.2 HCGI Incidence

The data were transformed to the highly credible gastrointestinal illness (HCGI) definition described previously (section 2.7.5).

Variations in the incidence of HCGI were observed during the observation period: for all groups the highest incidence was observed during autumn and winter 1993 and the lowest during the summer 1994 period. By autumn 1994, the incidence for all groups was slowly increasing again as expected for such illnesses. Similar observation were made for all subjects (Figure 16) and for children 2 to 12 years (Figure 17).

The relative risk was calculated using the RO group as the reference: data showed several peaks of excess incidence for the tap water consumers (Figure 19, Figure 18). Two peaks are of interest: the autumn 1993 and the spring 1994 events were observed during the early winter freeze and during spring thaw. These events affected all groups including the Plant group: this is plausible as all subjects did consume some tap water.

The average incidence of HCGI was in the expected range at 0.4 to 1.0 episodes/person-year, but it was higher during the first period (Figure 20, Table 16, Table 17, Table 18). For all subjects, the incidence was lowest in the RO and Plant groups at respectively 0.58 and 0.60 episodes/person-year. The rates were higher in the Tap-valve group at 0.70 episodes/person-year.

Variation in the incidence of HCGI was observed when the data was stratified by age, sex, status and area (Figure 20, Table 16, Table 17, Table 18). These observations were made for all subjects and for children 2 to 12 years old. The incidence was higher for female than for male subjects and it decreased with age. Proxy-reporting or self-reporting revealed a slight difference which is compatible with the fact that informants were mainly women in the 21 to 49 years old age group. The incidence was variable by geographical area, and the relative risk was generally higher for subjects in the regions further away from the plant. The July to December 1994 data did not reveal significant differences between groups except for children 2 to 5 years old in the Tap and Tap valve groups who were still experiencing a higher incidence of HCGI. As expected, the 2 to 12 years old age group had a higher incidence of illness than all subjects and children 2 to 5 years the highest. The differences between the groups were similar to those observed for the full dataset: children who were tap water consumers (Tap and Tap-valve) had a higher relative risk. Children from the Plant group had an apparently lower incidence of HCGI when compared to RO group subjects, an effect seen mainly for the later period of the study when the incidence was lower.

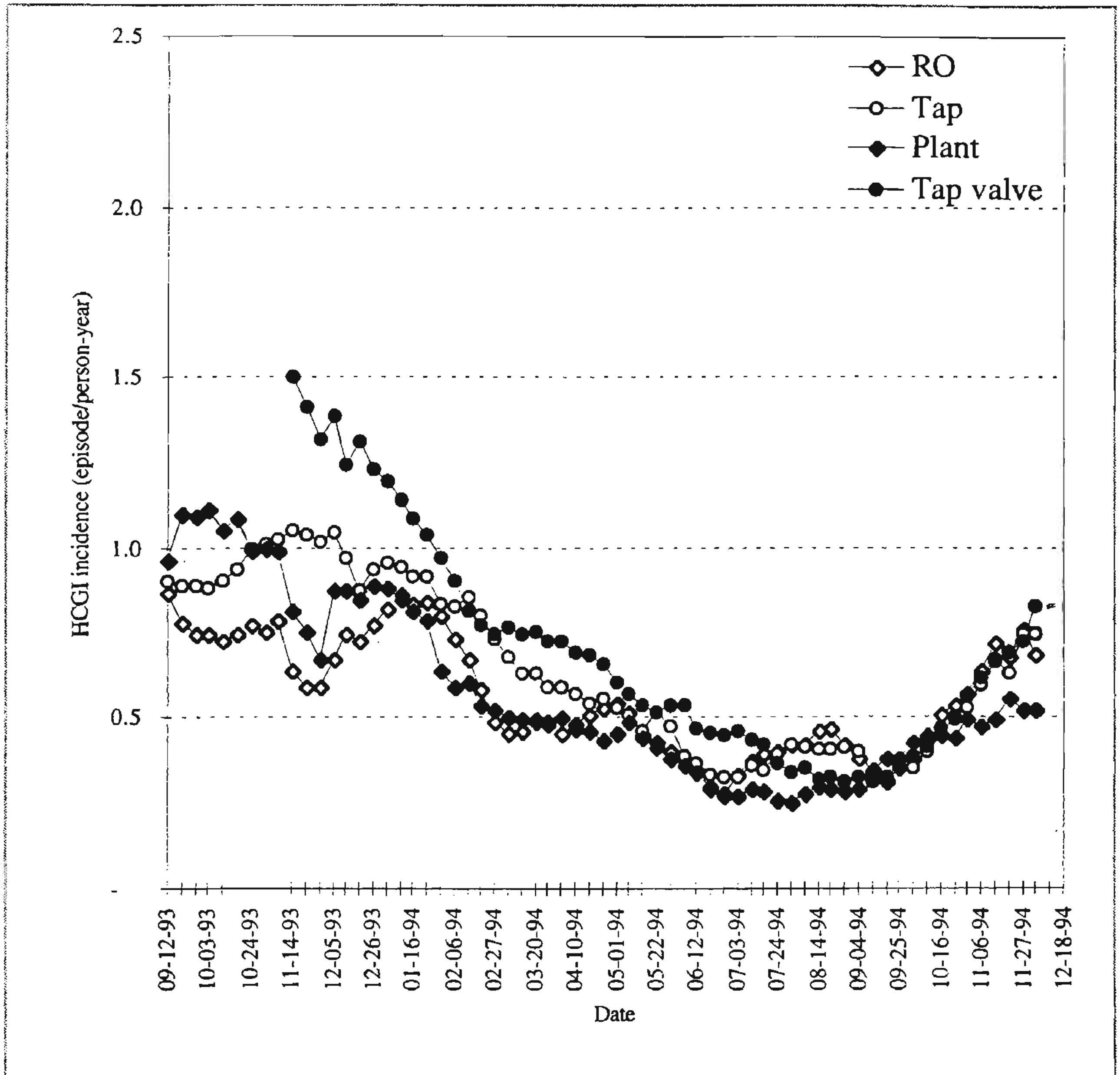


Figure 16 Rate of HCGI (8-week forward moving average) for all subjects in the four study groups.

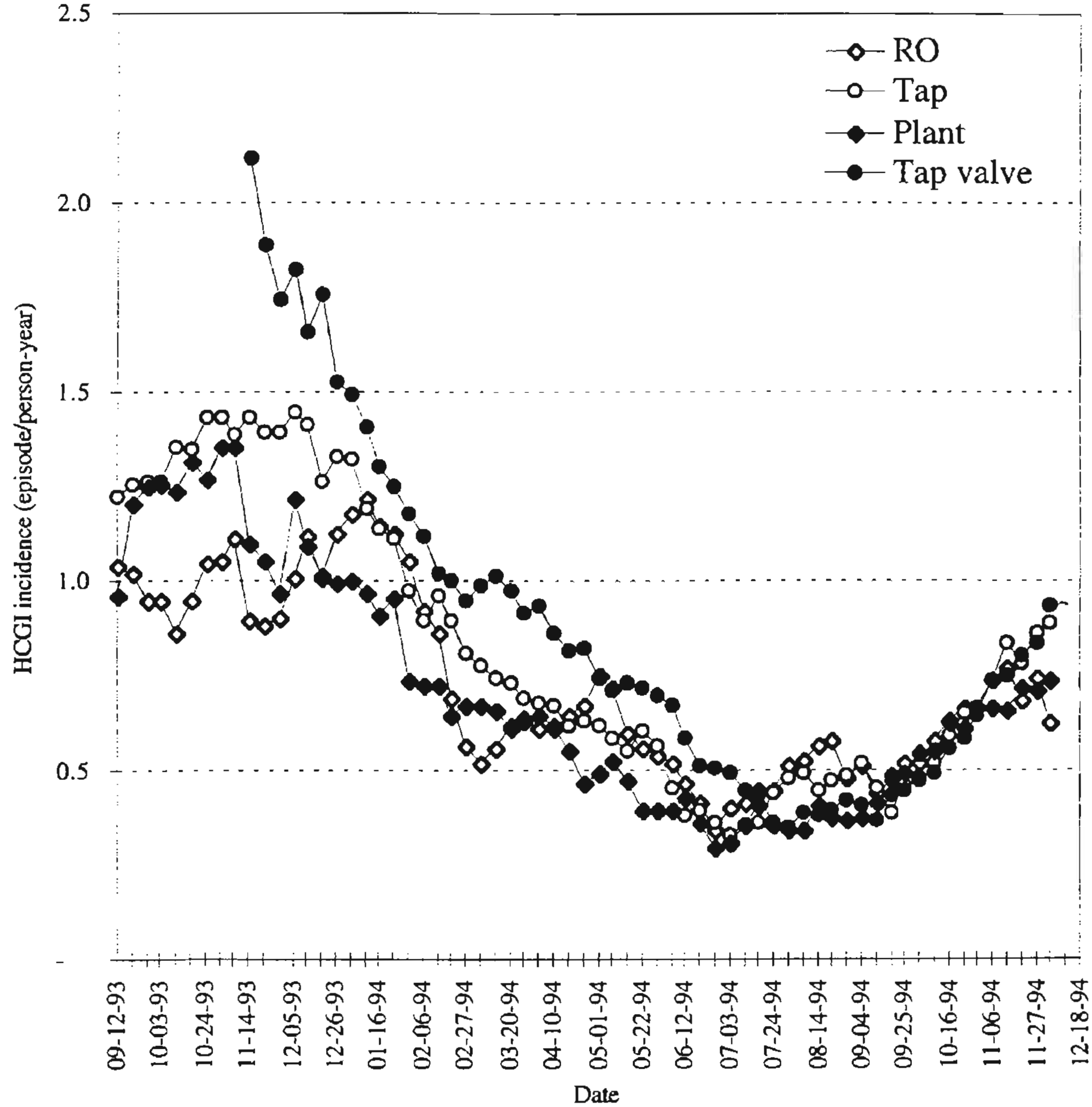


Figure 17 Rate of HCGI (8-week forward moving average) for children 2 to 12 years old in the four study groups.

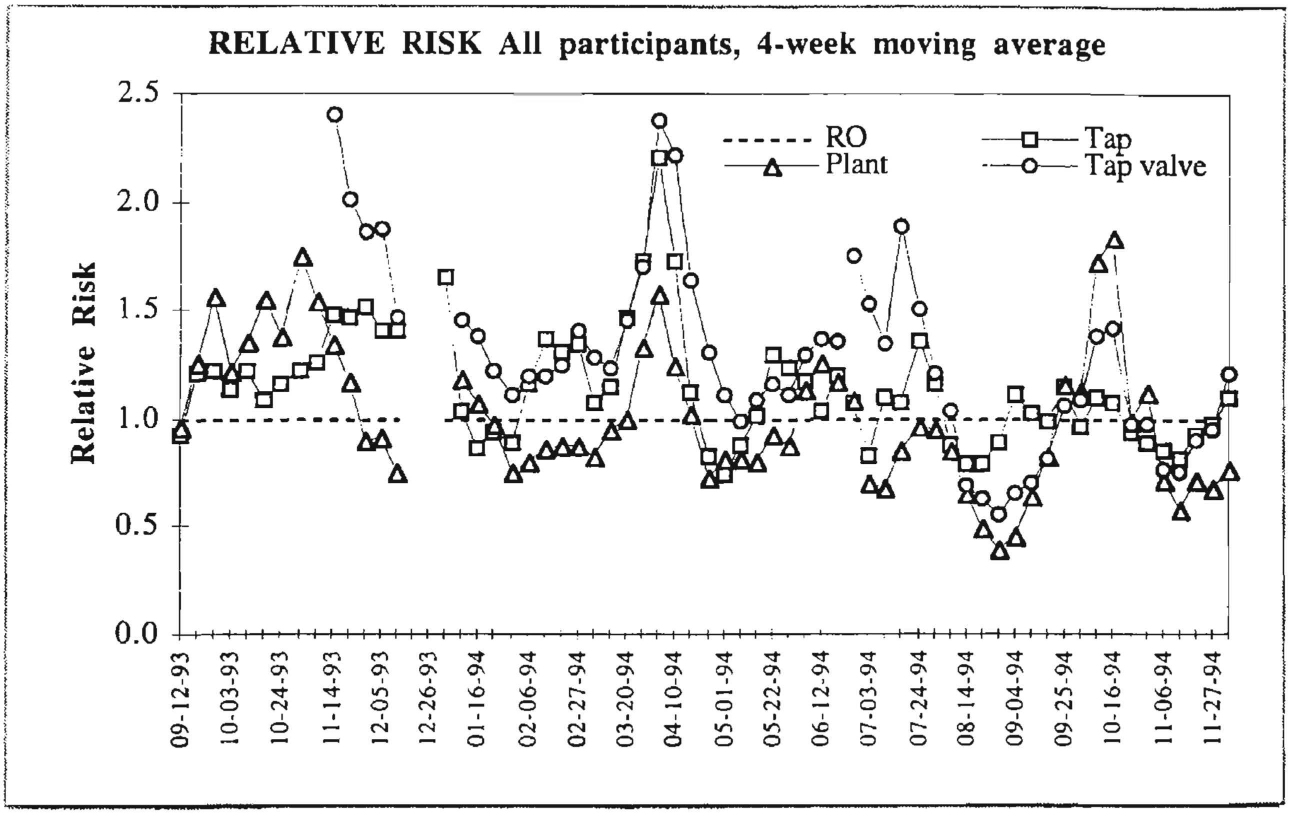


Figure 18. Relative risks for all subjects during the observation period.

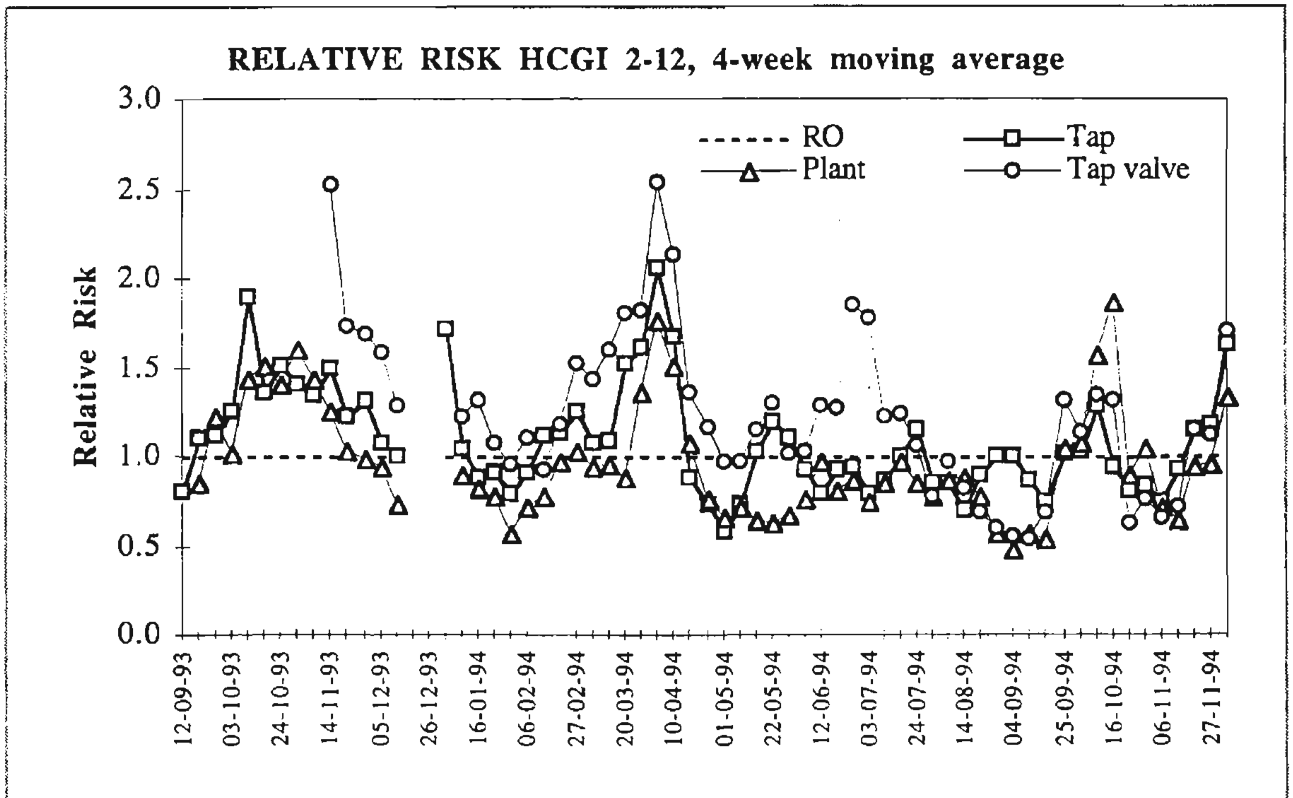


Figure 19. Relative risks for children 2 to 12 years old during the observation period.

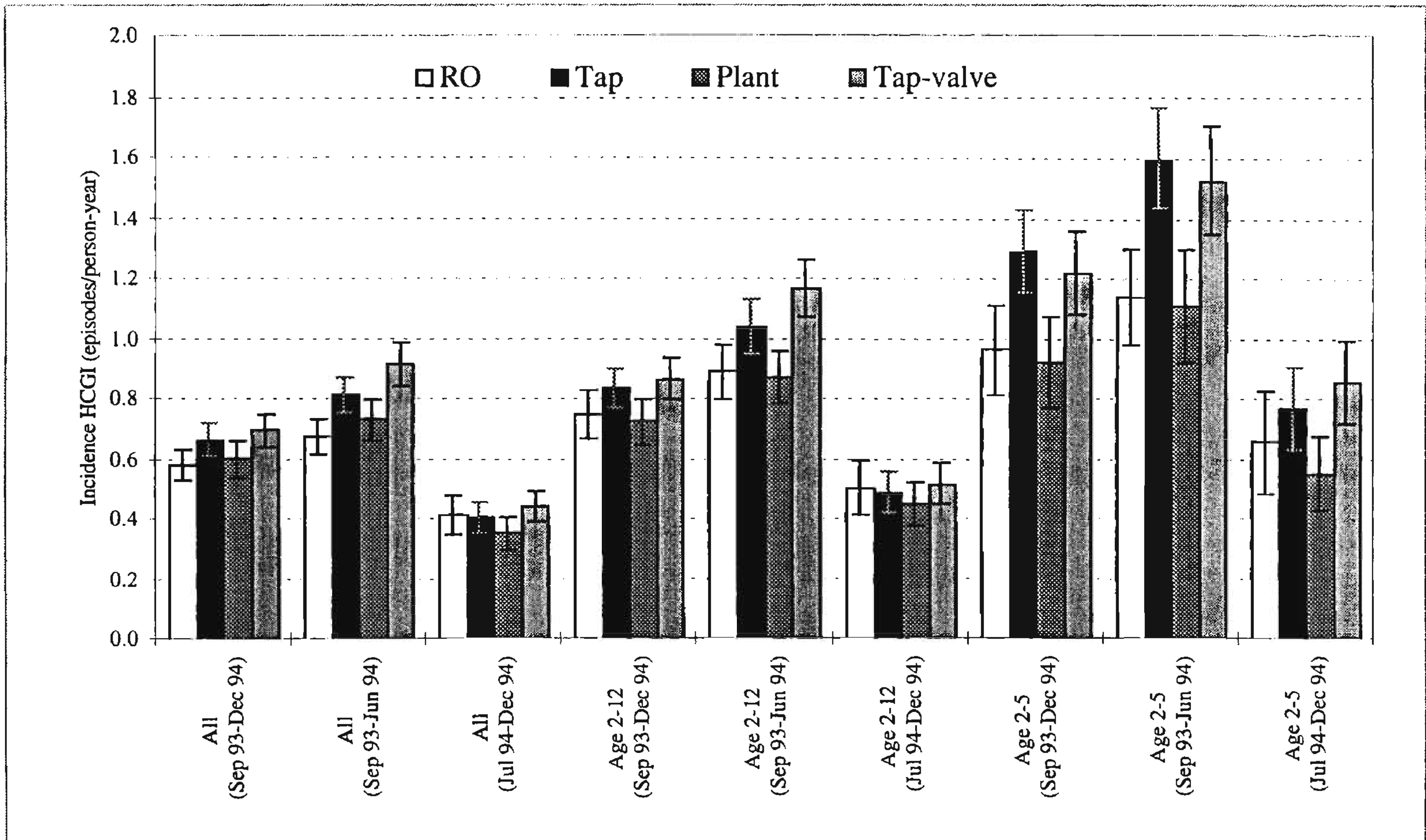


Figure 20. Comparison of HCGI rate by period and observation group for all subjects, children 2-12 years and 2-5 years old.

Table 16. Incidence and relative risks of highly credible gastrointestinal illnesses (HCGI) by group and calendar periods.

		RO			Tap			Plant			Tap-valve		
Sub-group	Period	Rate	s.e.	RR	Rate	s.e.	RR	Rate	s.e.	RR	Rate	s.e.	RR
All subjects	Period 1	0.68	0.06	1.0	0.81	0.06	1.19	0.73	0.07	1.07	0.92	0.07	1.35
	Period 2	0.42	0.06	1.0	0.41	0.05	0.98	0.36	0.05	0.86	0.44	0.05	1.05
	Period 1 and 2	0.58	0.05	1.0	0.66	0.05	1.14	0.60	0.06	1.03	0.70	0.06	1.21
Children 2 to 12 years	Period 1	0.89	0.09	1.0	1.04	0.09	1.17	0.87	0.09	0.98	1.17	0.09	1.31
	Period 2	0.50	0.09	1.0	0.49	0.07	0.98	0.45	0.07	0.90	0.52	0.07	1.04
	Period 1 and 2	0.75	0.08	1.0	0.84	0.06	1.12	0.72	0.07	0.96	0.87	0.07	1.16
Children 2 to 5 years	Period 1	1.14	0.16	1.0	1.60	0.16	1.40	1.11	0.19	0.97	1.53	0.18	1.34
	Period 2	0.66	0.17	1.0	0.77	0.14	1.17	0.55	0.12	0.83	0.86	0.14	1.30
	Period 1 and 2	0.66	0.17	1.0	0.77	0.14	1.17	0.55	0.12	0.83	0.86	0.14	1.30

Period 1 is from Sept. 93 to June 1994 and Period 2 from July to Dec. 1994
Rate number of episodes of highly credible gastrointestinal illnesses per person-year
s.e. standard error corrected for cluster sampling
RR Relative risk when compared to RO group.

Table 17. Incidence of highly credible gastrointestinal illnesses (HCGI) by group for all subjects for period 1 (September 1993 to June 1994).

		RO n = 1360			Tap n = 1296			Plant n = 1297			Tap-valve n = 1300		
	Subgroups	Rate	S.E.	RR	Rate	S.E.	RR	Rate	S.E.	RR	Rate	S.E.	RR
Age group	0 - 5	1.13	0.14	1.00	1.54	0.15	1.36	1.04	0.17	0.92	1.53	0.18	1.35
	6 - 20	0.73	0.07	1.00	0.78	0.07	1.07	0.74	0.08	1.01	0.91	0.08	1.25
	21 - 49	0.52	0.05	1.00	0.68	0.07	1.31	0.64	0.08	1.23	0.74	0.08	1.42
	50+	0.12	0.07	1.00	0.23	0.14	1.92	0.46	0.28	3.83	0.15	0.10	1.25
Sex	Male	0.63	0.06	1.00	0.78	0.07	1.24	0.68	0.08	1.08	0.81	0.08	1.29
	Female	0.72	0.07	1.00	0.85	0.08	1.18	0.78	0.08	1.08	1.02	0.09	1.42
Status	Informant	0.61	0.06	1.00	0.78	0.10	1.28	0.76	0.10	1.25	0.88	0.11	1.44
	Proxy	0.70	0.07	1.00	0.83	0.06	1.19	0.72	0.07	1.03	0.93	0.08	1.33
Area	Region 1	0.71	0.10	1.00	0.80	0.11	1.13	0.87	0.16	1.23	0.93	0.14	1.31
	Region 2	0.63	0.14	1.00	0.86	0.15	1.37	0.55	0.14	0.87	0.57	0.13	0.90
	Region 3	0.80	0.18	1.00	0.63	0.10	0.79	0.65	0.10	0.81	1.01	0.16	1.26
	Region 4	0.60	0.13	1.00	1.07	0.17	1.78	0.78	0.16	1.30	1.10	0.23	1.83
	Region 5	0.63	0.09	1.00	0.74	0.15	1.17	0.77	0.20	1.22	0.92	0.15	1.46
	All subjects	0.68	0.06	1.00	0.81	0.06	1.19	0.73	0.07	1.07	0.92	0.07	1.35

N Number of individuals
Rate number of episodes of highly credible gastrointestinal illnesses /person-year
s.e. standard error corrected for cluster sampling
RR Relative risk

Table 18. Incidence of highly credible gastrointestinal illnesses (HCGI) by group for children 2-12 years old for period 1 (September 1993 to June 1994).

Subgroups		RO n = 597			Tap n = 540			Plant n = 557			Tap-valve n = 561		
		Rate	S.E.	RR	Rate	S.E.	RR	Rate	S.E.	RR	Rate	S.E.	RR
Age	2 - 5	1.14	0.16	1.00	1.60	0.16	1.40	1.11	0.19	0.97	1.53	0.18	1.34
	6 - 12	0.79	0.08	1.00	0.85	0.08	1.08	0.77	0.09	0.97	1.01	0.09	1.28
Sex	Male	0.85	0.10	1.00	1.07	0.10	1.26	0.88	0.11	1.04	1.10	0.12	1.29
	Female	0.93	0.11	1.00	1.01	0.11	1.09	0.86	0.12	0.92	1.24	0.12	1.33
Area	Region 1	0.85	0.15	1.00	0.99	0.15	1.16	0.95	0.18	1.12	1.16	0.19	1.36
	Region 2	0.70	0.19	1.00	0.98	0.19	1.40	0.84	0.25	1.20	0.81	0.16	1.16
	Region 3	1.24	0.34	1.00	0.85	0.34	0.69	0.82	0.14	0.66	1.46	0.20	1.18
	Region 4	0.75	0.16	1.00	1.40	0.16	1.87	0.80	0.19	1.07	1.22	0.29	1.63
	Region 5	0.84	0.12	1.00	1.02	0.12	1.21	0.92	0.27	1.10	1.13	0.20	1.35
	All subjects	0.89	0.09	1.00	1.04	0.09	1.17	0.87	0.09	0.98	1.17	0.09	1.31

N Number of individuals
Rate number of episodes of highly credible gastrointestinal illnesses /person-year
s.e. standard error corrected for cluster sampling
RR Relative risk

3.5.2 Poisson regression analysis

Poisson regression analysis revealed that for all subjects and both periods combined, group membership was a significant predictor of the risk of HCGI (Table 19). The relative risk was 1.15 for the tap group and 1.24 for the tap valve group. Age was a significant variable, with children experiencing a higher incidence of HCGI. Significant differences were also observed for sex, observation periods and subregion. When the analysis was restricted to the informants, i.e. mainly females 21 to 49 years old, group differences were small. Among children however, whether youngest or all 2 to 12 years old, significant relative risks of 1.20 to 1.40 were associated with group membership. This effect was seen only in period 1.

Whenever group membership was significant, it was possible to verify simultaneously that there were no statistical difference between the two bottled water groups and the two tap water groups.

3.5.3 Correlation with water consumption and residence time

Significant dose-response relationships were observed between water consumption and incidence of HCGI for children and for informants in the Tap group (Figure 21, Table 20). In subjects over 12, including informants, the relationship was positive and in subjects less than 12 years the correlation was negative. Dose-response relationships were not observed when the data was analyzed for all subjects or for subjects in the Tap-valve group even if HCGI incidence was higher.

There was no correlation between the incidence of HCGI in a family and the time of residence of the water in the distribution system (data not shown).

3.5.4 Severity of events

Cold or flu was by far the most frequently reported symptom however, differences between the groups were small (Table 13). Diarrhea was frequently reported while bloody diarrhea, back pains, muscular pains and earaches were rarely reported. Changes in daily activities, absences from work or school, being bedridden and having to seek medical help were quite frequent, while hospitalization was a relatively rare occurrence in all groups. Subjects from the Tap-valve and Tap groups generally reported events more frequently than those in the RO or Plant group. Plant water consumers reported many of these events less frequently than subjects in the other three groups.

Table 19. Relative risks derived from Poisson regression analysis, by study group and unit of observation with age, sex, group participation and region as variables.

		Relative risk				p value for variable				
Period	Unit of observation	RO	Tap	Plant	Tap-valve	Group	Age	Sex	Region	Period
Sept. 93 - Dec. 94	All	1.00	1.15	1.00	1.24	0.007	0.000	0.001	0.000	0.000
Period 1 and 2	Informant	1.00	1.23	1.17	1.32	0.216	n.a.	0.028	0.002	0.000
	Youngest child*	1.00	1.24	0.99	1.29	0.147	0.000	0.805	0.032	0.000
	Children 2-12	1.00	1.14	0.94	1.21	0.079	0.000	0.797	0.102	0.000
Sept. 93 - Jun. 94	All	1.00	1.21	1.06	1.33	0.001	0.000	0.004	0.028	n.a.
Period 1	Informant	1.00	1.24	1.22	1.40	0.299	n.a.	0.106	0.090	n.a.
	Youngest child	1.00	1.29	1.04	1.39	0.039	0.000	0.963	0.162	n.a.
	Children 2-12	1.00	1.20	0.97	1.30	0.008	0.000	0.718	0.198	n.a.
Jul. 94 - Dec. 94	All	1.00	0.99	0.82	1.03	0.204	0.000	0.162	0.001	n.a.
Period 2	Informant	1.00	1.14	1.06	1.11	0.858	n.a.	0.190	0.020	n.a.
	Youngest child	1.00	1.06	0.83	1.00	0.800	0.000	0.587	0.265	n.a.
	Children 2-12	1.00	0.95	0.85	0.98	0.853	0.000	0.273	0.169	n.a.

n.a. not applicable
* Youngest children from group of 2 to 12 years old
p value for difference between groups

Table 20. Incidence of HCGI episodes by amount of water consumed among tap water subjects.

	Tap group					Tap valve			
	Glasses of water per week				Direction	Glasses of water per week			
Unit of observation	0 to 19	20 to 32	32+	p value*	of trend	0 to 19	20 to 32	32+	p value*
All subjects	1.03	1.07	1.03	0.975		1.90	1.74	1.68	0.226
Informant	0.38	0.60	0.69	0.005	Pos.	0.60	0.76	0.65	0.788
Child 2 to 12	1.30	1.04	0.96	0.020	Neg.	2.21	2.08	2.10	0.724
Subjects over 12	0.27	0.40	0.39	0.011	Pos.	0.42	0.38	0.36	0.279

Annual incidence derived by Poisson Regression model including age, sex and subregion.

* p value for Trend test. The trend was either positive (Pos.) or negative (Neg.).

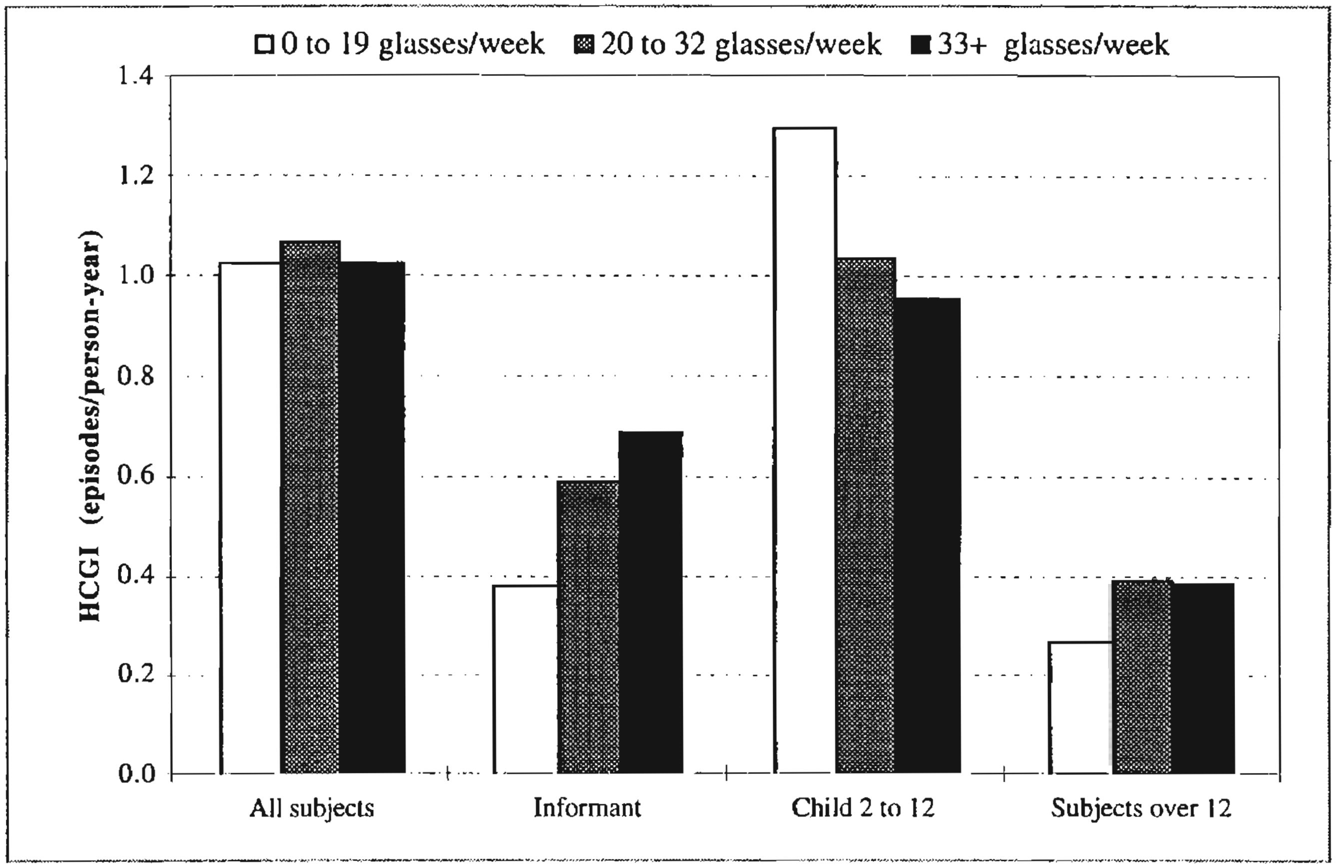


Figure 21. *Dose response analysis of water consumption and HCGI incidence in tap water cunsumers (Tap and Tap-valve groups).*

4. DISCUSSION

4.1 INCIDENCE OF ILLNESS

4.1.1 Are the current rates different from the previous study ?

The incidence of HCGI observed during the course of this study is very similar to the incidence observed during our earlier study (Payment et al. 1991b) (Figure 22, Table 22) and is comparable to the data reported in the early 1950's during the Cleveland studies (Hodges *et al.* 1956) and 1980's during the Tecumseh studies (Monto and Koopman 1980, Monto *et al.* 1983).

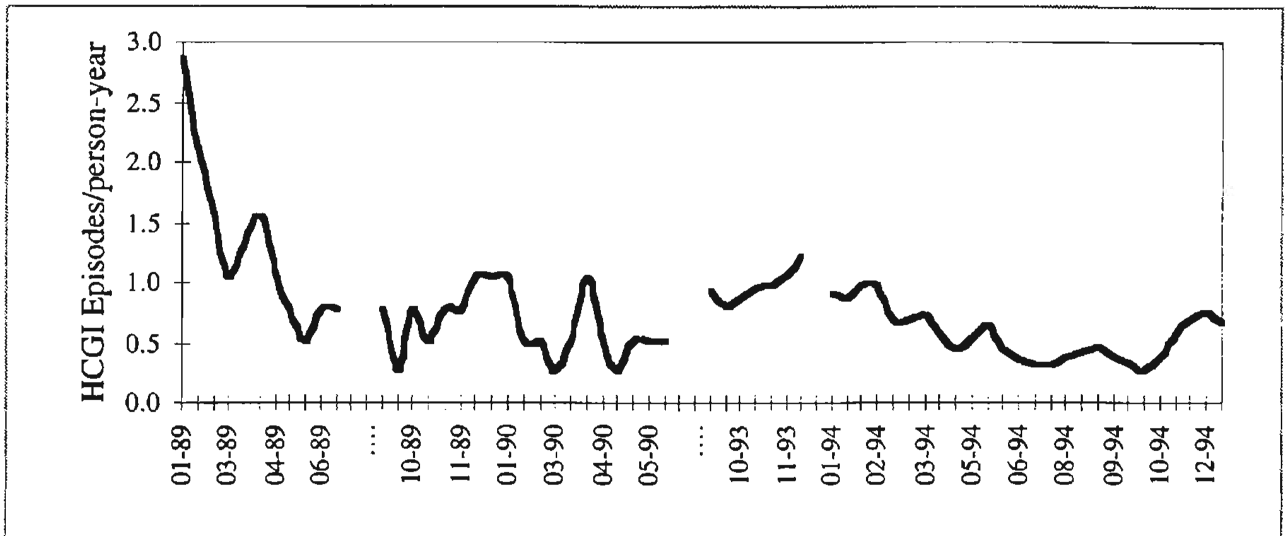


Figure 22 Comparison of HCGI bi-weekly rate for 1989-90 and 1993-94 data from the same area.

4.1.2 Why was the rate of HCGI higher at the onset of the observation period ?

A higher rate of reporting can be expected at the beginning of any observation period as subjects are probably eager to participate. This effect probably disappears after a few months and it could result in an apparently higher rate in the firsts weeks of observation. However, this should be equal for all groups: they were equally randomized and we are attempting to find a differential rate. Furthermore by selecting the most credible symptoms that are not easily misclassified by the subjects, we diminish the impact of this effect.

The reverse is also true, after almost a year of participation the level of reporting could be dwindling. By autumn 1994, we observed that the incidence was beginning to increase, indicating that the reporting system was still working. Finally, informal contacts with several of the families indicated that they were eager to participate and that the families were faithfully responding. As the questionnaires were returned to us we could also assess that reporting quality by comparing to the biweekly telephone interviews.

The annual average rate of respiratory and gastrointestinal illnesses is relatively constant from year to year, but the amplitude of weekly or monthly rates is relatively unpredictable from one year to the other due to large variations. During the first months of our 88-89 study (Payment *et al.* 1991b), the rate of gastrointestinal and respiratory illnesses in the population was 3 episodes/person-year while it was 1.5 in the current study (Figure 22).

In the course of any epidemiological study involving populations and over periods as short as 16 months cannot fully grasp the cyclical nature of these infections (Rotbart 1995). Outbreaks of many enteroviruses will reappear only every 5 to 10 years when the number of susceptible children is high enough to permit transmission. Enteroviruses are also known to be often implicated in respiratory syndromes (Rotbart 1995) and the fact that a proportion of the illnesses attributable to water were cold- or flu-like is not surprising in the current study.

4.1.3 What is the impact of a lower HCGI rate in the second observation period ?

The fact that a lower HCGI rate was observed during the second period had a negative impact on this study by diminishing the power of the statistical analyses. Because of the lower rate, we did not observe has many HCGI as expected but there were still enough observations to detect significant differences between the groups.

Table 21. Percentage of families with at least one HCGI episode during the 1988-89 and 1993-94 observation periods and groups.

Observation period	RO	Tap	Plant	Valve
Jan. 1988- June 89 (18 months)	62.0%	67.7%	ND	ND
Sept. 1993- Dec. 94 (16 months)	59.0%	65.4%	61.4%	64.4%

4.1.4 What percentage of the subjects were affected ?

In the current study, 59 to 65% of the families experienced at least one episode of HCGI over a period of observation similar to the one previously reported (Payment et al. 1991b). This was expected: families with more children and especially younger children will experience more HCGI than those with only one older child. The probability of being infected is such that it might take 2 or 3 years for some families before they experience any HCGI.

About 40% of the subjects experienced at least one episode of HCGI. This is also consistent with the probability of experiencing an HCGI when the average annual rate is about 0.5 episode/person-year and the observation that the HCGI rate is higher in children than adults. The number of HCGI observed is consistent with an observation period of 16 months.

4.1.5 What is the tap water related fraction of HCGI ?

Data collected during this study suggest that overall the tap water attributable level of HCGI varies from 14 to 35% when the data is stratified by period. Individual data of the relative risk suggest that it reached a RR of 2.5 (i.e. 250%) in the tap-valve group on two occasions (Figure 18). Still, this risk represents only part of the total burden of tap water-attributable illnesses because subjects in the bottled water groups were still exposed to some tap water. Tap water still accounted for about 40% of their drinking water. What we were able to do, by providing them with RO-treated water, was to reduce their exposure to tap water, not to eliminate it.

While the overall relative risk is 1.14 for the Tap group and 1.21 for the Tap-valve group (Table 16), the relative risks are respectively of 1.19 and 1.35 for the period of September 1993 to June 1994 (Table 16, Table 22). These values are generally lower than the overall relative risk of 1.5 reported previously (Payment *et al.* 1991b) (Table 22).

Table 22. Comparison of 1993-94 and 1988-89 HCGI data (Payment et al. 1991b).

		HCGI rate (episodes/person-year)				Relative Risk		
Age group	**Period**	RO	Tap	Plant	Valve	Tap	Plant	Valve
All	Jan. 88 -June 88	0.65	1.00			1.54		
	Sept. 88 -June 89	0.43	0.64			1.49		
	Sept. 93 -June 94	0.68	0.81	0.73	0.92	1.19	1.07	1.35
	July 94 - Dec. 94	0.42	0.41	0.36	0.44	0.98	0.86	1.05
0 - 5 years old	Jan. 88 -June 88	1.66	2.48			1.49		
	Sept. 88 -June 89	1.13	1.59			1.41		
	Sept. 93 -June 94	1.13	1.54	1.04	1.53	1.36	0.92	1.35
	July 94 - Dec. 94	0.64	0.84	0.48	0.90	1.31	0.75	1.41

4.1.6 Why is the water attributable fraction of HCGI lower in this study ?

The tap water-attributable fraction is lower in this observation period than in our first study. This was partly expected: the water treatment plant has been subjected to a major overhaul since the previous study was done and it thus expected that the quality of treatment would be enhanced significantly especially at the disinfection stage, both the ozonation and final chlorination.

A second explanation is the fact that subjects in the control group (RO-water) were drinking more tap water than during our first study: this has the overall effect of decreasing the detectable difference between the groups by increasing the HCGI rate in the control group.

4.1.7 What is the effect of tap water consumption by subjects in the bottled water groups ?

The 40% tap water consumption in the two bottled water groups is higher than our earlier data which had showed a 30% tap water consumption in these groups in our 1988-89 study (Payment *et al.* 1991b). In 1988-89, RO-filters had been installed under the kitchen sink of each household and RO-treated water was always available from a small tap located on the sink itself. In the current protocol, subjects had to physically get a 4-liter container of water from the refrigerator or the counter to obtain their drinking water. It appears that this procedure was more cumbersome and resulted in having many subjects get their water directly from the tap, a simpler procedure. As all subjects were originally tap water consumers (one of the criteria for enrollment), it appears that they often reverted to this source of drinking water. This has an **overall conservative effect,** as it reduced the difference that could be observed by exposing subjects in these groups to tap water contaminants. Any difference reported must be recognized as being an underestimation of any health effects. The total level of water related illness would have to be estimated to 50% if all tap water sources were taken in consideration.

4.1.8 Who is affected by water attributable HCGI ?

While some effect was seen in all age groups in the earlier study, current data suggest that the water attributable risk is higher in children consuming tap water. Children 0 to 5 years old have a relative risk of 1.31 to 1.49 (Table 22) if they drink tap water: half of their episodes of HCGI are thus due to tap water. The fact that the children are most affected is consistent with their developing immunity and susceptibility to pathogens.

Female subjects have a slightly higher incidence of HCGI, an observation that that is already known by the medical community. The water related fraction of these illnesses appear to be minor, generally less than 10% often with borderline significance for subjects in the Tap group. Female subjects in the Tap-valve groups were significantly affected while those in the Tap group were not

significantly affected suggesting that there is an effect but that it is probably less than 10%. The increased exposure due to the installation of the purge valve probably permitted the detection of this effect.

It is noteworthy that subjects in the Tap-valve group were affected whatever their age. Whether this is due to a higher exposure level or simply a chance observation remains to be shown.

4.1.9 Why was the rate of illness higher in the Tap-valve group ?

The fact that the subjects in the Tap-valve group had a higher rate of illness could be consistent with both a shortened residence time (decreased CT) and low bacterial regrowth in the distribution system and household pipes. These two factors need to be considered. The fact that water was flowing continuously in the households with purge valves implies that microorganisms, if present, would reach the consumer faster than if the water is left to stand in pipes for several hours. During stagnation, microorganisms would be exposed to microbial growth, a known inactivating factor for viruses (Hurst 1988a, 1988b, Cliver and Hermann 1972). They would also be exposed to leaching chemicals from pipes. Most of the tubing installed was copper and it has been reported that this type of pipes can inactivate viruses and bacteria (Abad *et al.* 1994, Yahya *et al.* 1991, 1992, Iquhoun *et al.* 1995). These explanations warrant further analysis.

Another hypothesis would be that the link created between the drain and the water line could have resulted in back-flow from the drain, exposing subjects to contaminated water. While this is possible, it would have resulted in a continuous effect that should have been seen even at later periods during the study. This was not the case and events in this group occurred at the same time as those in the tap group suggesting that the agents were in the water before it entered the household.

The fact that several valves were partly or completely closed until we went to investigate suggests that many subjects in this group were probably exposed at a level similar to those in the Tap-group. This is also a conservative factor which suggests that the effect of a valve is probably greater than estimated.

4.1.10 What is the contribution of the water treatment plant to the observed illnesses ?

We have observed events suggesting that the group receiving bottled plant water also experienced some effect as evidenced by the relative risk (Figure 19, Figure 20). The overall observations that we are reporting are a combination of multiple events that affected the subjects during those 16 months. Events in the Plant group (bottled plant water) could be explained by the fact that their water still contained some infectious agent resulting in the detection of a water attributable fraction in this group. On the other hand, subjects in the two bottled water groups, who were still consuming about 40% of their water as tap water, were exposed to contaminants whether they came from the plant or were introduced in the distribution system after treatment.

4.1.11 Was there any bias in reporting the symptoms ?

Bottling RO water and treated plant water was intended reduce reporting bias by attempting to prevent the subjects from identifying the water that were drinking. At the onset in the introductory letter they were only told that they would be receiving water of a different level of purity. Even if families would have attempted to compare their water, the bottles were identified in the same manner with only coded numbers. Blindness of the subjects to what water they consumed was thus achieved.

For subjects who drank plant, RO or spring water, it could be argued that their lower level of reported illness is due to the fact that they assumed that they were all drinking high quality water (i.e., "if it is bottled, it is better"). However, it is the group consuming plant water in which the perception of the quality of the water was toward "not always good". Furthermore, subjects in the Tap-valve group were the one who had the highest rate of illness but who considered their water as being of "very good quality" (i.e., continuously running water was less likely to have bad taste or odor).

4.1.12 What is the signification of the observations in the Plant (bottled water) group ?

Water obtained directly at the plant, before it entered the distribution system has not been affected by the distribution system. As the rate is lower in this group than in the Tap groups, this suggest that the tap water attributable illnesses probably originated from the distribution system.

Subjects in the Plant group, consuming mainly bottled plant water, were generally slightly less at risk that any of the other groups. While there is no statistical significance to this difference of about 10%, it remains an interesting observation as it is reflected in many reported events (Table 13). Because of the elevated bacterial regrowth observed in bottled plant water, we were somewhat worried that an increase in illness might result, but none was observed. During the 1989-90 study,

we had observed a correlation between disease and bacteria growing at 35°C in water from the domestic reverse-osmosis units (Payment *et al.* 1991a). It was thus quite surprising that in fact subjects in this group generally experienced no increase in illness and even had a lower rate of HCGI than the subjects in the RO water group. Bacteria detected in the RO units were mainly bacteria growing on the rubber substrates in the reservoir provided with these units. On the contrary, bacteria in the bottled plant water were bacteria common in tap water and in the distribution system.

4.1.13 Why would the distribution system be at fault ?

The fact that the rate of HCGI in the Plant group was equivalent or less than the RO group suggests that plant as it leaves the water treatment plant, is not the source of waterborne illness when properly treated as demonstrated in this study.

The distribution system and household plumbing are two possible sites that have been identified as potentially contributing microorganisms that could result in illnesses. Contaminated water entering the distribution system can carry pathogens (virus, parasites or bacteria) or opportunistic pathogens could multiply to numbers sufficient to induce disease.

The latter possibility, bacterial regrowth as the source of pathogens, is not supported by the current data. An attempt to correlate HCGI rate in the family with the residence time in the water distribution system did not reveal any correlation. This suggest that bacterial regrowth in the distribution system is probably not the cause for the illnesses observed: if it were, the longer residence time and the ensuing bacterial regrowth might have shown some correlation. While some regrowth did occur, the residence time did not exceed 25 hours, a relatively short period that would not result in high bacterial counts such as those encountered in the bottled plant water.

A distribution system is always subject to breaks or infiltration and repairs must be done on a regular basis. In a system such as the one under observation, infiltrations are usually not considered a major source of contaminants because the system is constantly under pressure. Breaks, major or minor, can however can be a source of contaminated material especially at the time of repairs. Even if the repairs are done according to the standards and properly disinfected, it is not known if microorganisms as resistant as protozoan cysts can survive. The elimination of coliforms might not provide a sufficient level of protection. Finally, the fact that the areas further away from the treatment plant were more at risk suggests distribution-related contamination. The protection afforded by residual chlorine would diminish with distance from the plant and slow decay of the disinfectant. Microorganisms entering the distribution system in breaks occurring closer to the plant would be subjected to a higher chlorine residual.

The fact that the subjects in the Tap-valve group had a higher rate of illness would be consistent with both a shortened residence time (decreased CT) and low bacterial regrowth in the distribution system and household pipes. These two factors need to be considered. The fact that water was running continuously in the households with purge valves implies that microorganisms, if present, would reach the consumer faster than if the water is left to stand in pipes for several hours. During stagnation, microorganisms would be exposed to microbial growth, a known inactivating factor for viruses (Hurst 1988a, 1988b, Cliver and Hermann 1972). They would also be exposed to leaching chemicals from pipes. Most of the tubing installed was copper and it has been reported that this type of pipes can inactivate viruses and bacteria (Abad *et al.* 1994, Yahya *et al.* 1991, 1992, Iquhoun *et al.* 1995). These explanations warrant further analysis.

4.1.14 What are the implications of tap water consumption in the bottled water groups

Subjects consuming the two bottled water groups were still drinking a significant proportion of tap water, accounting for about 40% of their water consumption (Figure 23). This is higher than our earlier data which had showed only 20 - 30% tap water consumption in the RO group during the 1988-89 study (Payment *et al.* 1991b). In 1988-89, RO-filters had been installed under the kitchen sink of each household and RO-treated water was always available from a small tap located on the sink itself. In the current protocol, subjects had to physically get a 4-liter container of water from the refrigerator or the counter to obtain their drinking water. It appears that this procedure was more cumbersome and resulted in having many subjects get their water directly from the tap, a simpler procedure. As all subjects were originally tap water consumers (one of the criteria for enrollment), it appears that they often reverted to this source of drinking water. This has an overall conservative effect, as it reduced the difference that could be observed by exposing subjects in these groups to tap water contaminants. Any difference reported here or earlier must be recognized as being an underestimation of any health effects. The actual level of water-related illness could be as high as 50%, if all tap water consumption is taken in consideration.

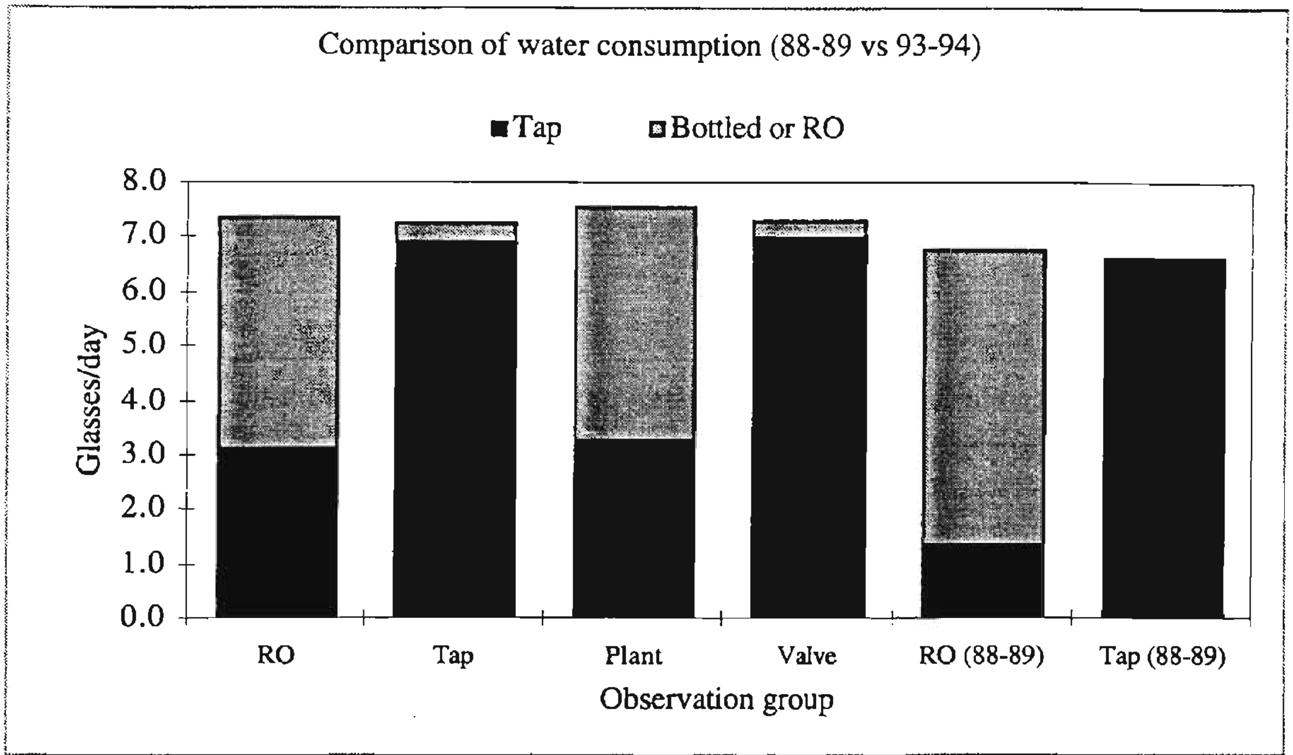


Figure 23 Frequency distribution of bottled water consumption for subjects by observation groups.

4.2 MICROBIOLOGICAL AND PHYSICO-CHEMICAL DATA

The water treatment plant was under scrutiny for the full period of this project. At all times, it met or exceeded current water quality requirements. Compliance with the coliform standard was 99.4% with 4/633 samples containing total coliforms. Fecal coliforms were never detected at any treated water sampling site and turbidity was close to 0.1 NTU with no values exceeding 0.28 NTU. There is no indications that any time during this study the plant was out of compliance.

The data collected at the plant showed that pathogens such as viruses and parasites numbers can vary significantly in river water. These variations were to be expected: many different virus types are circulating in the population and their daily number in water are the result of the number of individuals infected that day in the population (Rothbart 1995). Outbreaks, recognized or not, thus affect greatly the levels of viruses, bacteria and parasites in these waters.

Clostridium perfringens and somatic coliphages provided relatively constant indications of the level of pollution this water. Their numbers were relatively constant and they provided a means of estimating microorganisms removal by the water treatment plant. The water treatment plant achieved after filtration at least a 5 log removal of *Clostridium*, probably the most resistant indicator to treatment and disinfection. Coliphage, clostridial spores or human enteric viruses were

never detected in finished water suggesting the probable absence or very low numbers of live microorganisms.

We have no mean of estimating the viability of the cysts or oocysts that were observed in filtered water at the plant, but because they were observed later in the study when the rate of illness were in fact very low.

CT values calculated from the data obtained at the water treatment plant indicate that the plant was achieving at least a CT value of 82 mg/l.min in cold water and up to 221 mg/l.min in warm water during the summer period. Using the values provided by the USEPA, this is equivalent to 200 logs removal-inactivation of viruses, an unrealistic value. This is probably due to the fact that the models used for virus inactivation by the oxidants assume a linear dose response. For most microorganisms, this is not true and a more resistant fraction or a slower inactivation rate is often observed, especially when the curves are derived from data generated by field experiments.

The model predicts CT values that are quite conservative under very cold water situations. After filtration, a minimum 5 log removal was observed for somatic coliphages and *Clostridium perfringens*: the disinfection (ozone and chlorine at this plant) should then account for a much higher removal-inactivation value. Clostridial spores have been reported to be almost as resistant to disinfection as oocysts (personal communication, Mark Sobsey, University of North Carolina at Chapel Hill).

5. CONCLUSIONS

1) River water contained high levels of human enteric viruses, *Giardia* cysts and *Cryptosporidium* oocysts. None of these were detected in the treated water.

2) The water treatment plant produced water that met or exceeded current regulations and the distribution system was in compliance.

3) The plant was achieving at least a CT value of 82 mg/l.min in cold water and up to 221 mg/l.min in warm water during the summer period.

4) Subjects were equally randomized in the four study groups and represented an average socio-economic population.

5) Gastrointestinal illness in the population was within expected values at an average incidence of 0.6 episodes per person-year. The incidence was somewhat lower than expected during part of the observation period. Children experienced the highest incidence.

6) Consumption of drinking water was within expected values at an average of 7 glasses per day (about 1 liter). Subjects receiving bottled water still consumed about 40% of their drinking water as tap water a value slightly higher than expected but similar to what was observed in a previous study. This exposed subjects to potential contaminants from tap water and thus reduced the measurable difference between the groups.

7) Tap water consumers had an average excess of gastrointestinal illnesses of 14% (RR = 1.14) while in the Tap-valve group the excess was 21% (RR = 1.21). Subjects in the Tap-valve group had the highest risk levels. Given the fact that subjects in the RO group still consumed 40% of their drinking water as tap water, the correct values are probably higher than those measured.

8) Tap water attributable illness was not constant throughout the observation period: from September 1993 until June 1994, the excess risk was 19% (RR = 1.19) for subjects in the Tap group and 35% (RR = 1.35) in the Tap-valve group.

9) Children 2 to 5 years old were the most affected with an excess of 17% to 40% and the highest values observed from September 1993 to June 1994.

10) Increases in the relative risk during specific periods suggest that the overall observation is the result of several events during which the relative risk reached as high as 2.5 for children. These events occurred in Oct - Nov 1993 and in Mar- Apr. 1994. These periods coincide with winter freezing and spring thawing periods which could have resulted in breaches of the integrity of the distribution system and the occurrence of unreported outbreaks.

11) Plant water (bottled) was not the source of an overall excess of gastrointestinal illnesses suggesting that the distribution system was the major source of the observed effect.

12) Bottled plant water supported a significant bacterial regrowth: no health effects were associated with bacterial counts averaging more than 1,000,000 bacteria/100 ml (HPC at 25°C).

6. RECOMMENDATIONS

1) **In addition to a high quality treatment, the distribution systems should be monitored closely to evaluate their contribution of the previously unsuspected contribution to tap water related illnesses.**

In the recent years the focus has been placed on regulations aimed at the water treatment plant and the production of water that meets very high standards of quality: the water treatment plant under study met all these criteria and was operated at very high standards. The current observations have suggested that the distribution system and not the water treatment plant is the major source of gastrointestinal illnesses in tap water consumers. These occurrences were not detected by routine coliform monitoring.

2) **The protective effect of water stagnation and of copper pipes should be investigated.**

While the focus has been in reducing the level of bacterial regrowth to meet the coliform and chlorine residual standards, we might be eliminating an important purifying effect of bacterial regrowth and copper residuals in service lines and household plumbing. The installation of purge valves resulted in an unexpected excess of gastrointestinal illnesses: for now, two aforementioned hypotheses are the only explanations.

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ANNEXES

ID FAMILY:_______________ **Note:** If the person is absent more than one day, mark that day with an "X"

During the last 2 weeks, did anyone in your family had: **- cold or flu** **- vomiting** **- nausea** **- diarrhea**

If YES ( ) complete questionnaire for each person with symptoms

If NO ( ) do not complete the questionnaire

		Name:														Name:													
	Date	/1	/2	/3	/4	/5	/6	/7	/8	/9	/10	/11	/12	/13	/14	/1	/2	/3	/4	/5	/6	/7	/8	/9	/10	/11	/12	/13	/14
	SYMPTOM Day	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S
1	Nausea																												
2	Vomiting: How many times																												
3	Diarrhea: Number soft stools																												
4	Number liquid stools																												
5	Number liquid stools with blood																												
6	Fever Temperature in °F or °C																												
7	Abdominal cramps																												
8	Muscular pains																												
9	Back pains																												
10	Sore throat																												
11	Headache																												
12	Cold or flu																												
13	Earache																												

Because of these symptoms the person:

14	Changed daily activities																												
15	Was absent from work or school																												
16	Stayed in bed																												
17	Consulted MD																												
18	Was hospitalized																												

Taste of water during this period was Check (✓) appropriate word		Taste of water during this period was Check (✓) appropriate word	
Excellent	☐	Excellent	☐
Good	☐	Good	☐
Bad	☐	Bad	☐

Figure 24 Annex 1. Diary used to record the symptoms.

Water consumption questionnaire

NAME OF INDIVIDUAL:________________ ________________ -

FOR ONE DAY OF THE WEEK? (mark which one): Monday ☐ Tuesday ☐ Wdnesday ☐ Thursday ☐ Friday ☐

INDICATE THE NUMBER OF GLASSES CONSUMED

	AT HOME			OUT OF THE HOME		
TYPE OF BEVERAGE (NUMBER OF GLASSES)	Kitchen tap	Bathroom tap	Bottled water	Tap water	Bottled water	*Name of city where consumed*
WATER (as is)						
HOT WATER BEVERAGES (coffee, tea or other)						
COLD BEVERAGES (juices or drinks with cold water)						

FOR ONE DAY OF THE WEEKEND? (mark which one): Saturday ☐ Sunday ☐

INDICATE THE NUMBER OF GLASSES CONSUMED

	AT HOME			OUT OF THE HOME		
TYPE OF BEVERAGE (NUMBER OF GLASSES)	Kitchen tap	Bathroom tap	Bottled water	Tap water	Bottled water	*Name of city where consumed*
WATER (as is)						
HOT WATER BEVERAGES (coffee, tea or other)						
COLD BEVERAGES (juices or drinks with cold water)						

Figure 25 Annex 2. Questionnaire used to elicit water consumption data.