A Randomized, Blinded, Controlled Trial Investigating the Gastrointestinal Health Effects of Drinking Water Quality

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A double-blinded, randomized, controlled trial was carried out in in Melbourne, Australia, to determine the contribution of drinking water to gastroenteritis. Melbourne is one of the few major cities in the world that draws drinking water from a protected forest catchment with minimal water treatment (chlorination only). Six hundred families were randomly allocated to receive either real or sham water treatment units (WTUs) installed in their kitchen. Real units were designed to remove viruses, bacteria, and protozoa. Study participants completed a weekly health diary reporting gastrointestinal symptoms during the 68-week observation period. There were 2,669 cases of highly credible gastroenteritis (HCG) during the study (0.80 cases/person/year). The ratio of HCG episode rates for the real WTU group compared to the sham WTU group was 0.99 (95% confidence interval, 0.85–1.15, $p = 0.85$). We collected 795 fecal specimens from participants with gastroenteritis, and pathogens were not more significantly common in the sham WTU group. We found no evidence of waterborne disease in Melbourne. The application of this methodology to other water supplies will provide a better understanding of the relationship between human health and water quality. *Key words:* double-blind randomized trails, drinking water quality, gastroenteritis, waterborne disease. *Environ Health Perspect* 109:773–778 (2001). [Online 1 August 2001] *http://ehpnet1.niehs.nih.gov/docs/2001/109p773-778hellard/abstract.html*

The provision of high-quality drinking water is a fundamental element of good public health. Recently, a number of investigative studies and outbreaks of waterborne disease have raised concern about the safety of drinking water supplies in developed nations (*1*–*5*). Perhaps of most concern are two randomized but unblinded trials that suggested drinking water meeting conventional standards may cause between 14 and 34% of gastroenteritis in the community (*1*,*2*). In the United States this would imply that between 39 and 96 million cases of gastroenteritis each year are attributable to drinking water, based on a recent estimate of the incidence of gastroenteritis (*6*). The view that substantial endemic waterborne disease may exist is also supported by quantitative microbial risk assessment modeling data on *Cryptosporidium* oocysts in U.S. public water supplies, although there are many uncertainties in such estimates. (*7*).

These concerns have stimulated research on the impact of microbiological water quality on public health, as governments around the world seek to establish a sound scientific basis for water-quality regulation. This paper reports the results of the first randomized, double-blinded trial investigating waterborne disease. This trial was designed to determine whether microorganisms in a surface water supply with minimal treatment play a significant role in gastroenteritis in the community, thus providing an estimate of waterborne disease rates.

Methods

Setting and study description. The Water Quality Study was a double-blinded, randomized, controlled trial conducted in Melbourne, Australia, between September 1997 and February 1999. Six hundred families were randomly allocated to receive either real or sham water treatment units (WTUs) installed in their kitchen. Individuals used the water from these units as their main supply of drinking water during the study. The study received approval from the Monash University Standing Committee on Ethics in Research on Humans. Written informed consent was obtained from all adult participants and on behalf of child participants before participation in the study.

Ninety percent of Melbourne's drinking water is drawn from protected forest catchments with no farming, human habitation, or recreational activity. The area studied in this report draws all of its water from these catchments. The water is stored in large reservoirs for a minimum of 12 months before use and is chlorinated but not filtered before distribution through an enclosed system of storage tanks and pipes.

Eligibility and recruitment. To be eligible, families had to reside in the study area, have at least four eligible family members, including two children 1–15 years of age (as of 1 July 1997), and own or be purchasing their home. Home ownership was required, as the WTUs were plumbed into the household water pipes. The average rate of home ownership in Melbourne is > 73%, and the rate in the study area was > 80%. Each participant also had to consume at least one glass of tap water per day. Families with children were chosen because children experience higher rates of gastroenteritis than adults, thus increasing the statistical power of the study. Individuals were excluded if they were immunocompromised, had a chronic diarrheal illness, or were on long-term antibiotic therapy.

The study area contained 62,029 households, of which 10,918 met the demographic and home ownership criteria above. Invitations to participate were distributed by mail; through primary schools, child-care centers, maternal health centers, and shopping malls; and through advertisements in local newspapers. Interested families were invited to telephone the study center if they fulfilled the inclusion criteria. Eligible families were mailed a detailed information booklet and were then visited at home and enrolled in the study.

Definition of gastroenteritis. The primary end point of the study was highly credible gastroenteritis (HCG). The criteria for HCG were decided before data collection began. HCG was defined as any of the following symptoms in a 24-hr period: two or more loose stools, two or more episodes of vomiting, one loose stool together with abdominal pain or nausea or vomiting, or one episode of vomiting with abdominal pain or nausea. Cases of HCG were deemed to be distinct if the participant was symptom free for 6 days or more.

A less stringent definition of gastroenteritis, similar to the definition of gastroenteritis used in the Canadian studies (*1*,*2*), was also used in the analysis. The criteria for this secondary definition of gastroenteritis was any

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of the following symptoms in a 24-hr period: two or more loose stools, one loose stool together with abdominal pain or nausea, one or more episodes of vomiting, or an episode of abdominal pain with nausea. Cases were deemed to be distinct if the participant was symptom free for 6 days or more.

Data collection. Participants completed a health diary for each week during the 68-week observation period. Data collection was suspended for two intervals of 4 weeks corresponding to the Christmas (summer) holiday seasons. Each family had a reporting participant who was responsible for contact with study staff and ensuring that a health diary was completed for every family member each week. The health diary recorded details of gastrointestinal symptoms, medical treatment, and potential risk factors that may be associated with gastroenteritis (e.g., swimming, pet ownership). Diaries were returned by mail to the study center every 4 weeks. Diaries were then checked for completeness, and the reporting participant was telephoned to clarify missing or inconsistent answers. Health diaries were computer scanned, and the data files were imported into a Microsoft Access database (Microsoft Corporation, Redmond, WA, USA). Participants were not required to complete the health diary for days when they had been away from home overnight. Families were deemed noncompliant and were withdrawn from the study if they failed to return three sequential sets of diaries (12 weeks of data).

Participants estimated their water consumption on three occasions during the study. At the end of the study, we asked reporting participants whether they believed their WTUs were real or sham, or if they did not know the type.

Fecal specimens. Participants were asked to collect fecal specimens during episodes of gastroenteritis. The specimens were analyzed for *Salmonella* sp., *Shigella* spp., *Campylobacter* spp., *Vibrio* spp., *Yersinia* spp., *Aeromonas* spp., *Plesiomonas* spp., *Clostridium difficile* (culture and toxin), *Giardia*, *Cryptosporidium*, rotavirus, and adenovirus (*8*). Pathogenic *Escherichia coli* (defined as enteropathogenic, enteroaggregative, enterotoxigenic, enterohemorrhagic, and enteroinvasive types) were also characterized (*9*,*10*).

Water quality monitoring. Routine water quality monitoring was performed by the water utility from sampling points at customer properties. Samples were tested for fecal coliforms, total coliforms, and heterotrophic plate count (35–37°C) (*11*), and for free chlorine and total chlorine by standard methods (*12*).

A composite sample from the four water mains supplying the area was collected weekly and analyzed for selected pathogens. Protozoa were concentrated from a 400-L composite sample using a wound fiberglass cartridge filter (*13*). A fraction, equivalent to 150 L of water, was tested by reverse transcription-polymerase chain reaction (RT-PCR) for the presence of viable *Cryptosporidium parvum* oocysts (*14*) and viable *Giardia* species cysts (*15*). A second 150-L sample equivalent was purified on a percoll sucrose flotation gradient and examined by immunofluorescent microscopy (IMF) for the presence of *Giardia* cysts and *Cryptosporidium* oocysts using the Meridian Hydrofluor Combo detection system (EnSys Inc., Research Triangle Park, NC, USA). *Campylobacter* sp. were concentrated by filtration from a 5-L water sample followed by culture enrichment and detection by PCR (*16*). *Aeromonas* sp. were assayed by membrane filtration of 100-mL samples, and *Clostridium perfringens* spores by membrane filtration of 200-mL samples (*17*).

Water treatment units. The real WTUs consisted of a 1-µm absolute filter to remove protozoa and an ultraviolet (UV) unit to inactivate viruses and bacteria (Figure 1). To ensure effective blinding, the sham WTU was designed to appear identical to the real WTU, but had no filtration cartridge and a plain glass sleeve surrounding the UV source to prevent transmission of UV light into the water. Both the real and sham units warmed the water on standing. All units had a 5 L/min flow-limiting valve and a seal to prevent tampering. The WTUs were plumbed into the kitchen of each home and provided water through a separate tap.

The UV unit was a Steriflo SF300 (Contamination Control, Auckland, New Zealand), which met the disinfection performance specified in the ANSI/NSF STANDARD 55–1991 (class A, removing 99.9999% of bacteria and 99.99% of viruses) (*18*). The filtration unit was a Memtec Filterite Poly-Fine ARD1 filter (US Filter, Palm Desert, CA, USA) and met ASTM 795-62 (*19*) and ISO 4572 (*20*), removing 99.95% of 1-µm particles. The performance of the units was confirmed by challenge with *Cryptosporidium parvum* oocysts (filter cartridge), and *Klebsiella terrigena* ATCC 33257 (UV unit; American Type Culture Collection, Manassas, VA, USA). The filter cartridges and UV tubes in all units were replaced after 10–12 months operation. A random sample of 10 UV tubes removed after 12 months operation were tested for output to verify that UV dose remained above the required level (*18*).

Randomization and blinding of water treatment unit status. Households were allocated a real or sham WTU using a random number list. Participants and researchers were blinded to the type of the WTU allocated to each household. The WTUs were assembled and sealed at a workshop to ensure that the plumbers installing the WTUs were not aware of the unit type. When the units were serviced during the study, the entire WTU was removed and replaced by the same type of WTU without opening the unit at the participant's home. Unblinding of the researchers and families occurred after data collection was complete.

Sample size and statistical analysis. The primary analysis compared HCG event rates between the sham and filter group using the total number of HCG episodes for each individual over his/her observation time. To account for the correlation between the number of HCG events of individuals within the same family, we used generalized estimating equations (*21*). This implementation assumed an overdispersed Poisson model for individuals with an exchangeable correlation

Figure 1. The water treatment unit, which consisted of a 1-µm absolute polypropylene filter cartridge and an ultraviolet unit. Sham units appeared identical but were altered to have no microbiocidal effect. The units were installed under the kitchen sink, with treated water delivered through a separate tap.

Table 1. Baseline characteristics of participants.

Characteristic	Sham WTU	Real WTU
Number of families	300	300
Number of participants	1,399	1,412
Male	711 (50.8)	729 (51.6)
Age < 10 years	561 (40.2)	575 (40.9)
Attending child care	164 (11.7)	190 (13.5)
Highest level of education (adults)		
Trade	85 (14.4)	65 (10.8)
Secondary	352 (59.7)	381 (63.1)
Tertiary	153 (25.9)	158 (26.2)
Adults undertaking paid work	464 (74.5)	481 (77.7)

Numbers in parentheses indicate percentage.

structure within families and with individual observation time as an offset, and additionally adjusted for age, sex, and region of residence. Further analyses using the family as the analysis unit provided almost identical results and are not presented here.

This 68-week study was designed to detect a reduction of 15–20% in the overall rate of HCG episodes in the filter group with at least 80% power across a variety of parameter configurations using a two-sided 5% significance level. Assuming two adults and two children per family with annual event rates of 0.65 and 1.6 among adults and children, respectively, in the sham filter group, a total of 300 families were required. This calculation involved deriving the mean and variance of the number of HCG episodes per family and considered each individual's episode count as having an overdispersed Poisson distribution in which the variance exceeded the mean (with overdispersion factors ranging from 1.2 to 2.0), together with an intrafamily correlation in number of HCG episodes of up to 0.4. Maximum power to detect the smallest reduction is achieved if both the intrafamily correlation and the overdispersion are small—for example, 90% power to detect a 15% reduction when the intrafamily correlation is 0.2, and overdispersion factor is 1.2. All configurations above achieve at least 80% power to detect a 20% reduction.

Results

Participants were recruited between February 1997 and August 1997. In all, 74,770 invitation letters and 25,300 pamphlets were distributed, resulting in 1,489 enquiries from interested families. Information was mailed to 1,024 families, and 647 of these were eligible and willing to take part. Enrollment visits were made to 622 families, but 22 were excluded because they declined to take part or because it was not feasible to install a WTU in their home. The 600 families enrolled represented 5.5% of the families in the area who fitted the demographic and home ownership criteria.

The characteristics of the 600 families and 2,811 participants at randomization are shown in Table 1. Forty-one families (6.8%) withdrew from the study—20 from the real WTU group and 21 from the sham WTU group. The reasons for withdrawal in the real WTU groups were moving from study area, 11; divorce/family crisis, 4; noncompliance with health diaries, 2; serious illness, 1; kitchen renovation, 1; and disliked taste/odor of water, 1. For the sham WTU group, the reasons for withdrawal were moving from study area, 18; divorce/family crisis, 1; and noncompliance with health diaries, 2. A total of 173,298 person-weeks of health diary data were collected out of a possible 191,148 (90.7% returned).

There were 2,669 cases of HCG during the study, corresponding to an overall rate of 0.80 cases/person/year. There were 1,317 cases of HCG in those with real WTUs (0.79/person/year) and 1,352 cases in those with sham WTUs (0.82 /person/year; Table 2).

The ratio of HCG episode rates for the real WTU group compared to the sham WTU group was 0.99 [95% confidence interval (CI), 0.85–1.15; p = 0.85), with an estimated intrafamily correlation of 0.31 and overdispersion factor of 1.85. The HCG reporting rate decreased steadily over time; however, the relative effect of the real WTUs remained constant over time (p = 0.99 for interaction of group status with tertiles of observation time; Figure 2). The rate ratios were 0.94 (95% CI, 0.80–1.11; p = 0.49) for children younger than 10 years of age and 1.01 (95% CI, 0.83–1.23; p = 0.92) for individuals older than 10 years of age; these rate ratios were not significantly different (interaction p = 0.53; Table 2).

When the less stringent definition of gastroenteritis was used in the analysis, there were 1,754 cases of gastroenteritis in those with real WTUs (1.05/person/year) and 1,757 cases those with sham WTUs (1.06/person/year). The ratio of gastroenteritis episode rates for the real WTU group compared to the sham WTU group was 1.00 (95% CI, 0.87–1.16; p = 0.98).

Health diaries from 11 of the 68 data collection weeks were manually checked against the database. Of 513 HCG cases recorded on the database during these weeks, 1 case was found to be spurious (extraneous marking on health diary). Six probable cases were not recorded on the database due to incorrect completion of the health dairy by the participant (more than one answer filled in for a question requiring a single answer).

Laboratory tests of the WTUs demonstrated a 99.999% reduction in viability of *Klebsiella terrigena* spores by the UV unit,

Table 2. Distribution of cases of HCG among participants.

No. of HCG cases per person	All participants		Children < 10 years of age	
	Sham WTU (%) (n = 1,399)	Real WTU (%) (n = 1,412)	Sham WTU (%) (n = 561)	Real WTU (%) (n = 575)
0	682 (48.9)	705 (50.4)	203 (36.3)	216 (37.9)
1	371 (26.6)	378 (27.0)	149 (26.6)	171 (30.0)
2	193 (13.8)	176 (12.6)	109 (19.5)	94 (16.5)
3	75 (5.4)	64 (4.6)	54 (9.6)	44 (7.7)
4–5	55 (3.9)	57 (4.1)	32 (5.7)	36 (6.3)
6–9	18 (1.3)	16 (1.1)	13 (2.4)	8 (1.4)
≥ 10	1 (0.1)	3 (0.3)	0 (0)	1 (0.2)
Total cases	1,352	1,317	754	713

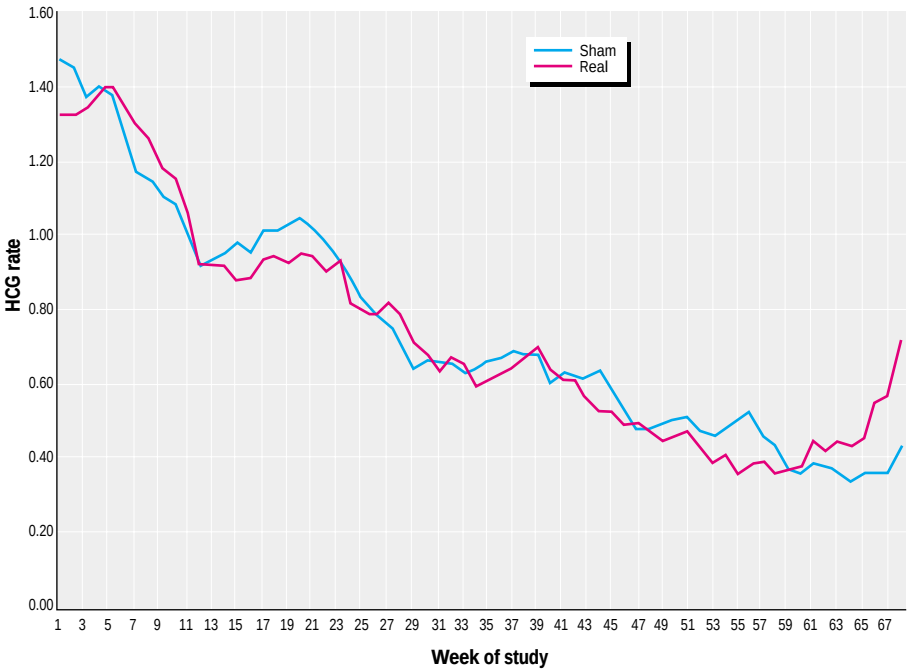

Figure 2. Rate of HCG (cases per person per year) during the study period in participants with real and sham WTUs (8-week moving average). Data collection was suspended for two intervals of 4 weeks during the main Christmas (summer) holiday periods (between weeks 14 and 15 and between weeks 62 and 63).

and no detectable passage of *Cryptosporidium parvum* oocysts after challenge by approximately 2,000 oocysts in 20 L of water. After 12 months of operation, the output from all of 10 randomly selected UV tubes remained above the required dose level.

Of the 559 families who completed the study, 520 (93%) replied to the blinding questionnaire. Reporting participants most commonly stated they were unable to guess the WTU type (Table 3). The number of reporting participants who correctly guessed their WTU type (n = 176) was slightly more than those who incorrectly guessed their WTU type (n = 133). Only 14% thought they had a sham WTU.

Participants consumed the majority of their unboiled water from the WTU. There was no significant difference in consumption of water from the WTU between the real and sham groups (mean 3.2 glasses/day and 3.4 glasses/day, respectively; p = 0.87). There was also no significant difference in the consumption of normal, unboiled tap water between the two groups (mean 0.55 glasses/day and 0.58 glasses/day, respectively; p = 0.32).

We collected 795 fecal specimens from participants with HCG. Pathogens were identified in 129 specimens (16.2%), with pathogenic strains of *Escherichia coli* being the most common organism (Table 4). Pathogens were not more significantly common in the sham WTU group.

Fecal coliforms were not detected in 1,167 routine 100-mL water samples collected in the water-quality zones that encompassed the study area. Total coliforms were detected in 219 samples (18.9%), with 63 samples (5.4%) containing more than 10 colony-forming units (CFU)/100 mL and the maximum count being 680 CFU/100 mL. Heterotrophic plate counts had a median of 37 CFU/mL, with 154 (13.2%) counts exceeding 500 CFU/mL.

Aeromonas sp. were detected in 50% of 68 weekly pooled mains samples, with positive samples having a median of 26 CFU/100 mL and a maximum of 710 CFU/100 mL. One sample was positive for *Campylobacter* sp. (week 43), and none were positive for *Clostridium perfringens* spores. Viable *Cryptosporidium parvum* oocysts were not detected in any of 68 weekly samples by RT-PCR. All samples were also negative for *Cryptosporidium* oocysts by IMF microscopy. Viable *Giardia* cysts were detected in two samples (week 44 and week 49) by RT-PCR, but *Giardia* cysts were not visualized by IMF microscopy, suggesting that only a few cysts were present in the 150-L sample equivalent analyzed.

Free chlorine levels in the distribution system ranged from 0 to 0.94 mg/L, with a median of 0.05 mg/L and 90% of samples having < 0.20 mg/L. Total chlorine levels ranged from 0.01 to 1.1 mg/L, with a median of 0.08 mg/L and 90% of samples having < 0.20 mg/L.

Table 3. Response to blinding questionnaire.

Reporting participant	Sham WTU	Real WTU
Thought WTU was real	105	129
Did not know	105	106
Thought WTU was sham	47	28

Discussion

There was no significant difference in the incidence of gastroenteritis in families with real or sham WTUs in our study. This demonstrates that waterborne pathogens do not play a major role in gastroenteritis in Melbourne because removal of microorganisms by point-of-use water treatment made no detectable difference to the rate of illness.

The low dropout rate and successful blinding of this study removes the concerns that hampered the interpretation of the two earlier randomized studies investigating water quality and health (*1*,*2*). The rigorous methodology established here will provide a useful tool to investigate the relationship between microbiological water quality and health in other water supplies.

The study was successfully blinded because only 43 (8%) more families correctly guessed their WTU type than incorrectly guessed their WTU type. This 8% difference is unlikely to have affected the validity of our results and is comparable to or better than other studies where the success of blinding has been reported (*22*–*24*). We avoided reverse osmosis or carbon filters because they change the taste of the water. The low turbidity and color of the water supply meant that the real WTUs had little effect on aesthetic characteristics.

The negative results of this study contrast with two previous unblinded randomized trials in Canada. In the first study, families consuming reverse osmosis (RO)-filtered water reported 34% less gastroenteritis than families consuming normal tap water (*1*). The second study had four water types: normal tap water, continuous running tap water, RO-filtered bottled water, and water bottled immediately after leaving the treatment plant. The rate of HCG was up to 19% higher in the tap water groups compared to the bottled water groups. The rates of gastroenteritis in the two bottled water groups were similar, but interpretation of the result was difficult because 50% of the bottled plant water group withdrew due to the taste and odor of the water, and the data recording period was not identical for all four groups (*2*). The unblinded nature of these studies means that the observed differences in gastroenteritis rates may have been partly or entirely attributable to reporting bias.

It should be noted the two Canadian studies (*1*,*2*) were performed in a community where the water supply was drawn from a heavily polluted river, although the water was subjected to conventional water treatment and filtration in addition to chlorination. Therefore, the difference in the results between the Canadian studies and our study may be due to the differences in the water supplies rather than differences in study methodology. The Melbourne catchment is unusually well protected from human and animal fecal pollution in comparison to catchments for most major cities in the developed world. Perhaps only a few cities in North America, for example, Portland, Oregon, and Seattle, Washington, could be considered as having comparable protected catchment areas.

In Melbourne, long detention times in large reservoirs also provide a substantial reduction in microbial content before chlorination and distribution to consumers. Nevertheless, the raw water cannot be regarded as pathogen free, as typically 45% of 100-mL prechlorination samples from the Silvan reservoir and 23% of samples from the Cardinia reservoir contain fecal coliform organisms. A recent survey of fecal coliform prevalence in raw water sources in 24 U.S. systems with a range of catchment types

Table 4. Pathogens identified in fecal specimens.

Pathogen	Number of positive specimens: All participants	Sham WTU	Real WTU	Relative risk (95% CI)	p-Value
Campylobacter spp.	24 (3.0%)	17	7	0.45 (0.2–1.1)	0.09
Escherichia coli (pathogenic)	53 (7.5%)	17	36	2.05 (1.1–3.7)	0.02
Salmonella sp.	9 (1.1%)	6	3	0.53 (0.1–2.1)	0.37
Adenovirus	9 (1.1%)	6	3	0.52 (0.1–2.0)	0.34
Rotavirus	11 (1.4%)	7	4	0.53 (0.2–1.8)	0.31
Cryptosporidium sp.	13 (1.6%)	9	4	0.47 (0.1–2.5)	0.37
Giardia sp.	20 (2.5%)	8	12	1.59 (0.6–4.0)	0.32

The following numbers of specimens were tested: all participants, 795; sham WTU group, 399; and real WTU group, 396. Ten specimens had two pathogens identified. Pathogenic *Escherichia coli* were defined as enteropathogenic, enteroaggregative, enterotoxigenic, enterohemorrhagic, or enteroinvasive strains. Pathogens not identified in any fecal specimens were *Aeromonas* spp., *Clostridium difficile* (culture and toxin), *Shigella* spp., *Vibrio* spp., *Plesiomonas* spp., and *Yersinia* spp.

reported an average of 65% of samples were fecal coliform positive (*25*).

The reported rate of HCG fell during our study from 1.37 cases/person/year during the first 13 weeks to 0.43 cases/person/year during the last 13 weeks, but the decrease was the same in the real and sham WTU groups. We believe this reflects underreporting of gastroenteritis symptoms due to declining motivation of participants. However, we cannot exclude the possibility that our groups experienced a real decline in gastroenteritis, perhaps due to behavioral changes in personal hygiene or food handling as a consequence of being involved in a study of this nature. Decreases were also observed in reporting of other health-related questionnaire items (respiratory symptoms, medical consultations, medication use), but not in lifestyle items (travel, presence of household pets; data not shown). This tends to support the former explanation for the decline in reported gastroenteritis rates. Similar declines have been seen in other studies (*1,2*), and it may be advisable for future studies to use a shorter observation period with a larger number of participants.

The lower confidence interval around our point estimate of 0.99 indicates that up to 15% of gastrointestinal disease in Melbourne could be caused by drinking water. However, this is unlikely for several reasons, including the high degree of similarity in the distribution of gastroenteritis episodes in both groups throughout the study period and the absence of any history of recognized waterborne outbreaks in Melbourne. The majority of pathogens isolated during episodes of gastroenteritis were chlorine-sensitive organisms, which are unlikely to have been transmitted via a chlorinated water supply. Most of the *Cryptosporidium* isolates identified during the study were attributable to a swimming pool-related outbreak (*26*).

Use of a less stringent definition of gastroenteritis resulted in a 32% increase in the number of recorded gastroenteritis episodes and also showed no significant difference between real and sham WTU groups. However, there was minimal effect on the size of the confidence interval around the point estimate as the majority of extra episodes captured by this definition occurred in participants who had experienced episodes under the original definition of HCG. This indicates that a substantial increase in participant numbers would be required to achieve a more precise estimate of effect.

During our study, total coliform bacteria were detected in the water more frequently than is recommended by the World Health Organization Guidelines for Drinking Water Quality (*27*), the Australian Drinking Water Guidelines (*28*), and the relevant U.S. standards (*29*). These bacteria are used as general indicators of water quality and may include organisms from both fecal and nonfecal sources. In Melbourne, total coliform bacteria are not detected immediately downstream of chlorination points nor at the outflow of suburban storage tanks; however, they are frequently detected at sampling points in the distribution system. It is believed this represents bacterial growth in the system rather than survival of organisms from the source water.

The negative result of our study confirms previous observations that the presence of total coliforms is not necessarily associated with disease risks (*30*). Therefore, increasing the disinfectant residual level in the distribution system in Melbourne simply to reduce the total coliform count may not be beneficial to community health. The role of high disinfectant residuals in distribution systems and their efficacy in protecting public health is a topic of some controversy in the international water industry (*31*). In the United States, relatively high free chlorine residuals (> 0.1 mg/L) are generally maintained in chlorinated systems, while in some European countries no residual is used after primary disinfection (*32*).

The importance of waterborne disease, both epidemic and endemic, and the need to establish better understanding of its occurrence and magnitude, has been recognized internationally. The U.S. Environmental Protection Agency is undertaking a research program that includes trials of a similar design to the Melbourne study (*33*), and the Organisation for Economic Co-operation and Development and the World Health Organization have recently established an international Expert Working Group to develop best practice approaches to this issue.

The successful completion of the Melbourne Water Quality Study with effective blinding and a low dropout rate demonstrates that rigorous epidemiological methods can be used to assess the impact of water quality on human health. The application of the study methodology to other water supplies will provide health and water authorities with a better understanding of waterborne disease and its contribution to endemic gastroenteritis. This will allow communities and regulators to make better informed and more cost-effective decisions about future water treatment requirements.

References and Notes

1. Payment P, Richardson L, Siemiatycki J, Dewar R, Edwardes M, Franco E. A randomized trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. Am J Public Health 81:703–708 (1991).
2. Payment P, Siemiatycki J, Richardson L, Renaud G, Franco E, Prevost M. A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. Int J Environ Health Res 7:5–31 (1997).
3. MacKenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N Engl J Med 331:161–167 (1994).
4. Furtado C, Adak GK, Stuart JM, Wall PG, Evans HS, Casemore DP. Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992-5. Epidemiol Infect 121:109–119 (1998).
5. Willocks L, Crampin A, Milne L, Seng C, Susman M, Gair R, Moulsdale M, Shafi S, Wall R, Wiggins R, et al. A large outbreak of cryptosporidiosis associated with a public water supply from a deep chalk borehole. Commun Dis Public Health 1:239–242 (1998).
6. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. Food-related illness and death in the United States. Emerg Infect Dis 5:607–625 (1999).
7. Regli S, Odom R, Cromwell J, Lustic M, Blank V. Benefits and costs of the IESWTR. J Am Water Works Assoc 91:148–158 (1999).
8. Hellard ME, Sinclair MI, Hogg GG, Fairley CK. Prevalence of enteric pathogens among community based asymptomatic individuals. J Gastroenterol Hepatol 15:290–293 (2000).
9. Paton AW, Paton JC. Detection and characterization of shiga Toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{0111}, and rfb_{0157}. J Clin Microbiol 36:598–602 (1998).
10. Robins-Browne RM, Yam WC, O'Gorman LE, Bettelheim KA. Examination of archetypal strains of enteropathogenic *Escherichia coli* for properties associated with bacterial virulence. J Med Microbiol 38:2222–2226 (1993).
11. Standing Committee of Analysts. The Microbiology of Water 1984. Part 1 - Drinking Water:Methods for the Examination of Waters and Associated Materials. Report on Public Health and Medical Subjects No 71. London:HMSO, 1984.
12. Eaton AD, Clesceri LS, Greenberg AE, eds. Standard Methods for Examination of Water and Wastewater. Washington, DC:American Public Health Association, 1995.
13. Kaucner C, Stinear T. Sensitive and rapid detection of viable *Giardia* and *Cryptosporidium parvum* in large volume water samples using wound fibreglass cartridge filters and reverse transcription PCR. Appl Environ Microbiol 64:1743–1749 (1998).
14. Stinear T, Matusan A, Hines K, Sandery M. Detection of a single viable *Cryptosporidium parvum* oocyst in environmental water concentrates by reverse transcription-PCR. Appl Environ Microbiol 62:3385–3390 (1996).
15. Mahbubani MH, Bej AK, Perlin M, Schaefer FW, Jakubowski W, Atlas RM. Detection of *Giardia* cysts by using the polymerase chain reaction and distinguishing live from dead cysts. Appl Environ Microbiol 61:3456–3461 (1991).
16. Eyers M, Chapelle S, Van Camp G, Goossens H, De Wachter R. Discrimination among thermophilic *Campylobacter* species by polymerase chain reaction amplification of 23S rRNA gene fragments. J Clin Microbiol 31:3340–3343 (1993).
17. Standing Committee of Analysts. The Microbiology of Water 1994. Part 1 - Drinking Water: Methods for the Examination of Waters and Associated Materials. Report on Public Health and Medical Subjects No 71. London:HMSO, 1994.
18. NSF International. Ultraviolet Microbiological Water Treatment Systems. ANS/NSF 55-1991. Ann Arbor, MI:NSF (National Sanitation Foundation) International, 1991.
19. ASTM. Standard Practice for Determining the Performance of a Filter Medium Employing a Single-Pass, Constant-Rate, Liquid Test. ASTM F795-62. West Conshohocken, PA:American Society for Testing and Materials, 1983.
20. ISO. Hydraulic Fluid Power–Filters–Multi-Pass Method for Evaluating Filtration Performance. ISO 4572-1981(E). Geneva:International Organisation for Standardization, 1981.
21. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized models. Biometrika 73:13–22 (1986).
22. James KE, Bloch DA, Lee KK, Kraemer HC, Fuller RK. An index for assessing blindness in a multi-centre clinical trial: disulfiram for alcohol cessation - a VA cooperative study. Stat Med 15:1421–1434 (1996).

23. Byington RP, Curb JD, Mattson ME. Assessment of double-blindness at the conclusion of the beta-Blocker Heart Attack Trial. JAMA 253:1733–1736 (1985).
24. Noseworthy JH, Ebers GC, Vandervoort MK, Farquhar RE, Yetisir E, Roberts R. The impact of blinding on the results of a randomized, placebo-controlled multiple sclerosis clinical trial. Neurology 44:16–20 (1994).
25. Nieminski EC, Bellamy WD, Moss LR. Using surrogates to improve plant performance. J Am Water Works Assoc 92:67–78 (2000).
26. Hellard ME, Sinclair MI, Black J, Dharmage SC, Bailey M, Andrews RM, Kirk MD, Fairley CK. An outbreak of cryptosporidiosis at an urban swimming pool: why are such outbreaks difficult to detect. Aust NZ J Public Health 24:272–275 (2000).
27. WHO. Guidelines for Drinking-Water Quality. Health Criteria and Other Supporting Information, Vol 2. Geneva:World Health Organization, 1996.
28. NHMRC/ARMCANZ. Australian Drinking Water Guidelines, 1996. Canberra, Australia:National Health and Medical Research Council: Agricultural and Resource Management Council of Australia and New Zealand, 1996.
29. U.S. EPA. Current Drinking Water Standards: National Primary and Secondary Drinking Water Regulations. Washington, DC:U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water, 1999.
30. Zmirou D, Ferley JP, Collin JF, Charrel M, Berlin J. A follow-up study of gastro-intestinal diseases related to bacteriologically substandard drinking water. Am J Public Health 77:582–584 (1987).
31. Clement JAM. The disinfectant residual dilemma. J Am Water Works Assoc 91:24–30 (1999).
32. Haas CN. Benefits of using a disinfectant residual. J Am Water Works Assoc 91:65–69 (1999).
33. U.S. EPA. Waterborne disease studies and national estimate of waterborne disease occurrence. Fed Reg 63:42849–42852 (1998).