Long-term Health Sequelae Following *E. coli* and *Campylobacter* Contamination of Municipal Water

Population Sampling and Assessing Non-participation Biases

Amit X. Garg, MD1,2 Jennifer Macnab, PhD1,2 William Clark, MD1 Joel G. Ray, MD, MSc3 John K. Marshall, MD, MSc4

Rita S. Suri, MD1 P.J. Devereaux, MD4,5 Brian Haynes, MD, PhD4,5 on behalf of the Walkerton Health Study Investigators

**ABSTRACT**

**Background:** Following bacterial contamination of a municipal water system in the rural town of Walkerton, Ontario, over 2,300 cases of acute gastroenteritis were documented. The Walkerton Health Study is currently underway to assess for long-term health sequelae among consenting inhabitants of Walkerton, related to the original outbreak. We explored whether the association between the acute exposure and preliminary long-term health outcomes may have been biased through differences between early- and late-recruited study participants.

**Methods:** Using multiple data sources, including the 1996 and 2001 Canadian Census, and records from the Regional Health Unit, hospital and Walkerton Health Study, we determined both sample representativeness and the anticipated effects of intensifying study participant recruitment. Selection bias was assessed by examining for differences between initial and late participants, and their subsequent risk of having hypertension, proteinuria and reduced renal clearance.

**Results:** Of the 4,315 participants, 2,756 were permanent residents of Walkerton, representing 55% of the town’s total population. The sample was demographically similar to the population of interest, although statistically women were more likely to participate than men (55% of sample were women compared to 52% of population, \( p < 0.01 \), and the proportion of both young and very elderly adults was smaller than expected (13% of sample were ≥65 years of age compared to 18% of population, \( p < 0.01 \)). Comparing the initial 3,959 participants to the 356 persons additionally recruited with substantial effort, the latter were more likely to be free of symptoms during the outbreak (21% vs. 7%, \( p < 0.001 \)), but were otherwise similar in terms of age, sex, the use of medical care resources and underlying health state predating the outbreak. The risk of long-term hypertension or renal sequelae did not significantly differ between initial and late study recruits.

**Conclusions:** Participants in the Walkerton Health Study represent the population of interest, and comprise those who were acutely ill during the infected water outbreak. The available study sample should provide reasonably unbiased estimates of the associated risk between acute bacterial gastroenteritis and long-term health sequelae.

**MeSH terms:** Cohort studies; epidemiologic studies; water supply; sampling studies; selection bias; *Escherichia coli* O157

La traduction du résumé se trouve à la fin de l’article.

1. Division of Nephrology, University of Western Ontario, London, Ontario
2. Department of Epidemiology and Biostatistics, University of Western Ontario
3. Department of Medicine, University of Toronto, Toronto, Ontario
4. Department of Medicine, McMaster University, Hamilton, Ontario
5. Department of Clinical Epidemiology and Biostatistics, McMaster University

**Correspondence:** Dr. Amit Garg, Kidney Clinical Research Unit, Room A01, Westminster Tower, London Health Sciences Centre, 800 Commissioners Road East, London, ON N6A 4G5, Tel: 519-685-8502, E-mail: Amit.Garg@lhsc.on.ca

**Acknowledgements:** Supported by: Ontario Ministry of Health, Kidney Foundation of Canada, Canadian Diabetes Association, and the Canadian Institutes of Health Research (CIHR). Drs. Garg and Marshall were supported by CIHR Clinician Scientist Awards. Dr. Devereaux was supported by a CIHR Senior Research Fellowship. Special thanks to Ms. B. Sackett and Dr. D. Sackett for advice and support. Other Walkerton Health Study investigators include Dr. S. Collins, Dr. J. Howard, Dr. J. Mahon, Dr. D. Matsell, Dr. L. Moist, Dr. J. Pope, Dr. P. Rosos-Avellano and Dr. M. Salvadori.

Walkerton is a small rural town located in one of Ontario’s prime agricultural areas, 180 kilometres northwest of Toronto. In May 2000, municipal water in Walkerton became contaminated with *Escherichia coli* O157:H7 and *Campylobacter* species. At the time of the outbreak, heavy rainfall contributed to the surface transport of livestock fecal contaminants into inadequately chlorinated drinking water, supplied from three drilled wells. Many individuals were either primarily or secondarily exposed to the bacteria, including permanent residents of Walkerton, individuals employed in the town, and visitors from neighbouring communities. This outbreak produced an estimated 2,300 cases of acute gastrointestinal illness, over 750 emergency room visits, 65 hospital admissions, 27 cases of identified hemolytic uremic syndrome (HUS), and 6 fatalities.1

While initial medical care focussed on the acute management of infected persons, attention has now shifted towards long-term health matters. Previous biological and observational studies suggest that survivors of *Campylobacter* gastroenteritis and severe *E. coli* O157:H7 infection may have poorer long-term health, regardless of whether they initially experienced overt HUS. Initiated in 2002, the Walkerton Health Study provides a unique and rare opportunity to characterize new and existing epidemiological associations between acute gastroenteritis and chronic diseases, including post-infectious irritable bowel syndrome, arthritis, hypertension, renal disease, and diabetes mellitus.2-7 Additionally, any psychosocial sequelae of this stressful environmental disaster may be better characterized.8,9 As part of the Walkerton Health Study, we invited citizens from the region to attend a special clinic in the local hospital, to undergo a standardized interview, physical examination, and blood and urine testing.

Since more severe acute illness is often associated with worse long-term outcomes,6 self-selection bias may lead to the erroneous finding of either no association, or an over-estimate of the association, between previous gastroenteritis and subsequent chronic disease. For example, those who decide to attend a study health clinic or who choose to volunteer for a study may differ from non-participants (non-respondents). Non-participation may be due to denial, selec-
tive migration or differences in health-seeking behaviours. As a group, if volunteers differ from non-participants in their association between acute illness and long-term sequelae, then the measure of association may be distorted, and the generalizability of the results threatened.10

Here, we assessed for study sample representativeness, and explored whether the association between the acute exposure and preliminary long-term health outcomes may have been biased by differences between early study participants and those who agreed to participate after more intense recruiting efforts.11

METHODS

We posed the following three questions: 1) Were study volunteers similar in age and sex to the general population of Walkerton and the wider municipality of Brockton? 2) What proportion of those who were acutely ill during the contaminated water outbreak agreed to participate in the long-term study? 3) What was the effect of intensifying efforts at participant recruitment? For the latter, we examined for differences between initial and late recruits, in terms of demographics, medical conditions predating the outbreak, and acute illness characteristics. Additionally, we explored whether the associated risk between having acute gastroenteritis and certain subsequent health measures differed between early and late study respondents.

Data sources

Both the 1996 and 2001 Canadian Census counted individuals in relation to their usual place of residence. According to the Census, non-commuter rural communities experienced a population decline due to both an emigration of young adults and the death of aged residents.12 Similarly, the municipality of Brockton, Ontario, which includes the town of Walkerton, experienced a 5% population decline over this five-year period. At the time of the outbreak in May 2000, Census, local postal and voting records suggested that there were 5,000 persons living in the town of Walkerton, and 10,000 persons in the municipality of Brockton, a land area of 565 square kilometres (Figure 1). A formal investigation at the time of the outbreak by the Regional Health Unit identified all individuals who were acutely ill by considering the following: i) persons who contacted the Regional Health Unit directly, ii) those who underwent laboratory stool cultures, and iii) all emergency room visits and admissions to any of the three local hospitals in Owen Sound, Walkerton and Hanover, Ontario.1 A total of 1,346 cases were identified from a history of diarrhea, positive stool cultures or recognized hemolytic uremic syndrome. The Regional Health Unit also conducted a random sample telephone survey at the time of the outbreak, to calculate age- and sex-specific attack rates for the town of Walkerton in the month of May 2000. By applying these estimates to age- and sex-specific population estimates provided by the 1996 Canadian Census, it was concluded that only 58% of cases had likely been identified. Accordingly, it was estimated that about 2,300 area residents had, in fact, been acutely ill at the time of the outbreak.

Beginning in February 2002, individuals who either lived in the Walkerton area or who had consumed municipal water in May 2000, irrespective of whether they developed an acute illness, were invited to participate in the Walkerton Health Study through word of mouth, the local media (radio and television), mailed leaflets, and a town hall meeting. Subsequently, in 2003, a detailed recruitment and telephone campaign was conducted, inviting non-participants from Walkerton to attend the health clinic in the local hospital. Posters with recruitment targets were placed in the post office and pharmacies, and 1,147 non-participating households were called using the local phone book. Both initial and additional participants were given a small honorarium of $10 to $20 Canadian for travel costs. In addition, satellite clinics were set up in the local high school and nursing home. Data obtained at the time of the standardized interview, physical assessment and laboratory testing were entered into a computer database by trained staff unaware of the acute exposure status of the participant.

Using pre-specified algorithms, persons with abnormal results were flagged for
TABLE I
Age- and Sex-distribution Data of Residents of Walkerton and Surrounding Areas Compared to the 1996 and 2001 Canadian Census Data

<table>
<thead>
<tr>
<th>Municipality of Brockton</th>
<th>Municipality of Brockton</th>
<th>Town of Walkerton</th>
<th>Town of Walkerton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10,163</td>
<td>9,655</td>
<td>5,036</td>
</tr>
<tr>
<td>Female (%)</td>
<td>52.0</td>
<td>50.1</td>
<td>52.0</td>
</tr>
<tr>
<td>Age years (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>6.6</td>
<td>5.0</td>
<td>6.6</td>
</tr>
<tr>
<td>5-14</td>
<td>15.3</td>
<td>14.0</td>
<td>13.3</td>
</tr>
<tr>
<td>15-19</td>
<td>6.6</td>
<td>9.0</td>
<td>6.6</td>
</tr>
<tr>
<td>20-24</td>
<td>5.9</td>
<td>5.2</td>
<td>5.9</td>
</tr>
<tr>
<td>25-54</td>
<td>39.0</td>
<td>40.8</td>
<td>39.0</td>
</tr>
<tr>
<td>55-64</td>
<td>8.8</td>
<td>10.6</td>
<td>8.8</td>
</tr>
<tr>
<td>65-74</td>
<td>8.5</td>
<td>8.2</td>
<td>8.5</td>
</tr>
<tr>
<td>≥75</td>
<td>9.2</td>
<td>7.3</td>
<td>9.2</td>
</tr>
</tbody>
</table>

These data refer to permanent residents. The age of the Walkerton Health Study participants was according to May 1, 2000.

The sample was demographically similar to the population of interest, although statistically women were more likely to participate than men (55% of sample were women compared to 52% of population, p<0.01), and the proportion of both young and very elderly was smaller than expected (13% of sample were 65 years of age or older compared to 18% of population, p<0.01).

TABLE II
Illness Characteristics of Walkerton Health Study Participants and Non-participants in Relation to the Initial Outbreak of Water-contaminated Escherichia coli O157:H7 and Campylobacter Species

<table>
<thead>
<tr>
<th></th>
<th>Participants</th>
<th>Non-participants</th>
<th>All Persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. individuals with recognized hemolytic uremic syndrome*</td>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>No. individuals with acute gastroenteritis confirmed by the Regional Health Unit</td>
<td>848</td>
<td>587</td>
<td>1346</td>
</tr>
<tr>
<td>No. individuals with self-reported acute gastroenteritis</td>
<td>1893</td>
<td>407</td>
<td>2300 †‡</td>
</tr>
<tr>
<td>No. individuals with stool cultures performed during outbreak</td>
<td>571</td>
<td>104</td>
<td>675</td>
</tr>
<tr>
<td>No. individuals with positive E. coli O157:H7 stools during outbreak</td>
<td>134</td>
<td>42</td>
<td>176</td>
</tr>
</tbody>
</table>

* Subsequent to the initial investigation, at least 3 more cases of hemolytic uremic syndrome have been identified. Of the 10 non-participants, 6 died within weeks of the outbreak.
† Estimated by the Regional Health Unit.
‡ The true number of individuals who drank contaminated municipal water or who were secondarily exposed to infection is unknown.

Based on self-reported and laboratory-confirmed definitions of acute illness, between 63% and 85% of all those identified as having been acutely ill are current participants in the study.

By February 2004, 4,315 study participants were recruited, of whom 2,756 (64%) were residents of Walkerton and 3,124 (72%) were residents of the wider municipality of Brockton. This represents about 55% of all Walkerton inhabitants and 31% of Brockton inhabitants at the time of the initial outbreak.

Study participants from Walkerton and Brockton were similar in age and sex to the Canadian Census figures for 1996 and 2001 (Table I), with the exception of a smaller proportion of young and very elderly adults (13% of sample were ≥65 years of age compared to 18% of population, p<0.01), and a higher representation of women (55% of sample were women compared to 52% of population, p<0.01). Demographic differences between the Census and study sample were consistent between population Census data and those of the Walkerton Health Study participants. Chi-squared or Fisher’s Exact tests were used to assess for differences between initial and subsequent volunteers. Relative risks for long-term renal sequelae after acute gastroenteritis and their 95% confidence intervals were calculated and graphed on a logarithmic scale. Statistical significance was set at a p-value <0.05. All analyses were conducted using SPSS 10.0 (SPSS, Inc., Chicago, IL, USA) and Excel 2000 (Microsoft Corporation, Redmond, WA, USA). All participants provided written informed consent, and the study was approved by the University of Western Ontario Research Ethics Board.
in those with and without gastrointestinal symptoms at the time of the outbreak (data not shown).

Specific characteristics of Walkerton Health Study participants and non-participants at the time of the contaminated water outbreak are presented in Table II. Based on self-reported and laboratory-confirmed definitions of acute illness, between 63% and 85% of all those identified as having been acutely ill are current participants in the study.

Following the 2003 campaign, an additional 356 initial non-participants were successfully recruited from Walkerton (Table III). Compared to initial participants, additional recruits were more likely to have been symptom-free at the time of the outbreak (21% vs. 7%, p<0.001), middle-aged (p<0.01) and have a family history of hypertension (35% vs. 42%, p<0.01). Otherwise, they were comparable to those recruited soon after the contaminated water outbreak (Table III).

Of the 4,315 study participants, 907 had evidence of hypertension or renal disease pre-dating the outbreak. Of the remaining participants, 1,135 described no symptoms at the time of the outbreak, 773 had confirmed acute gastroenteritis, and 1,500 were suspect cases. While recognizing the relatively small number of additionally-recruited participants, their risk of hypertension and renal sequelae necessitating nephrological consultation was similar to that of the early recruits (Table IV and Figure 2). The inclusion of the additional study participants to the whole study sample did not change the overall risk of long-term sequelae following acute gastroenteritis (Figure 2).

**DISCUSSION**

The Walkerton Health Study was designed to identify whether persons infected with *E.coli* O157:H7 or *Campylobacter* species are at extended risk of adverse health outcomes. For the purpose of screening and prognosis, this requires that the association between acute infection and long-term sequelae be characterized accurately. While a "target population" usually refers to those individuals to whom a study is applicable, it is difficult and/or impractical to study an entire population, even after a fixed and relatively contained outbreak, like that which occurred in Walkerton. After such an unpredictable event, establishing methods of collecting clinical and laboratory data on more than 4,300 participants living in a remote community requires funding, planning and time. In the case of Walkerton Health Study, two years had elapsed before the first participant was enrolled. As such, inferences must be drawn from a convenience sample of voluntary and consenting participants, who may differ from non-participants according to important baseline and prognosticating variables. The data presented herein suggest that, in a small and willing community, and using a variety of baseline sampling and ongoing recruitment strategies, study participants do appear to be representative of the population of interest. While those recruited through additional efforts were less likely to have been acutely ill during the outbreak, they were otherwise comparable to those recruited soon after the outbreak. Thus, non-participation has not significantly biased estimates of certain health conditions that predated the exposure of interest.

A true measure of non-participation bias is usually not possible, since non-participants cannot be assessed on how they differ from study volunteers in the relationship between an acute exposure and a long-term outcome. A large uncontrolled municipal water outbreak is particularly challenging in this regard, as the true number of exposed individuals remains unknown, and the methods of establishing a comprehensive sampling frame of eligible participants and non-participants remains elusive, often referred to as 'non coverage'. Nevertheless, our results establish a framework by which selection biases can be partially assessed in this health study, by comparing associations in those who initially volunteered to those who were recruited only after a substantial effort. This commonly used technique is imperfect, since for late recruits a longer period of time has elapsed between the initial exposure and the first study contact and collection of baseline and current data. There will also always be a fixed number of late recruits, and in some cases a limited sample size may provide inade-
Among Initial Participants and Those Additionally Recruited Through a Telephone Campaign

**TABLE IV**

Incidence of Newly Diagnosed Hypertension, Proteinuria or Reduced Glomerular Filtration Rate Prompting Nephrology Referral Among Initial Participants and Those Additionally Recruited Through a Telephone Campaign

<table>
<thead>
<tr>
<th></th>
<th>Initial Participants (n = 1773)*</th>
<th>Additional Participants (n = 135)*</th>
<th>Total Participants (n=1908)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Symptoms at the Time of the Outbreak</td>
<td>Confirmed Gastroenteritis at the Time of the Outbreak</td>
<td>No Symptoms at the Time of the Outbreak</td>
</tr>
<tr>
<td>No.</td>
<td>1026</td>
<td>747</td>
<td>1135</td>
</tr>
<tr>
<td>No. (%)*</td>
<td>42 (4.1%)</td>
<td>28 (3.7%)</td>
<td>45 (4.0%)</td>
</tr>
</tbody>
</table>

* Those with pre-existing hypertension or renal disease, and those with suspected but unconfirmed gastroenteritis, were excluded from this analysis.

Appreciating the relatively small number of additionally-recruited participants, the risk of hypertension and renal sequelae necessitating nephrological consultation in additionally-recruited participants did not statistically differ from that of early recruits.

**Relative Risk** (95% confidence interval)

| Risk lower | 0.1 |
| Risk higher | 0.4 |

**Figure 2.** According to those recruited initially and subsequently, the risk of hypertension, proteinuria or reduced glomerular filtration rate 2 years after acute symptomatic gastroenteritis.

Appreciating the relatively small number of additionally-recruited participants, the risk of hypertension and renal sequelae necessitating nephrological consultation in additionally-recruited participants did not statistically differ from that of early recruits.

The limitations related to small sample sizes and a follow-up period of three years or less, there was no overt difference in the risk of long-term hypertension and renal disease after acute gastroenteritis between early and late participants. The inclusion of another 356 late recruits did not change the overall associated risk of detectable long-term sequelae in the entire study sample. Thus, we believe that the Walkerton Health Study sample will provide reasonably unbiased estimates of the risk of long-term disease after acute bacterial gastroenteritis. In the future, these selection analyses will be repeated, and will include appropriate imputation methods and sensitivity scenarios on the number of individuals who would need to be recruited to meaningfully change the observed association. Ongoing public health efforts will continue to ensure that medical sequelae can be identified, treated and prevented in those who remain non-participants.

In health surveys, eligible non-participants may be heterogeneous in nature. A change in the strength or direction of various associations due to non-participation may depend on the nature of the health survey, the sampling method used, and the uniformity of non-participant variables relative to those of enrollees. As observed by others, we found that non-participants were more likely to be male and elderly, and had less symptoms of acute illness. Others have suggested that non-participants are also less likely to be formally educated or employed, more likely to engage in smoking, poorer lifestyle behaviours and medication use, while receiving fewer routine health care visits and having less health care expenditures.

In the special situation of a contained environmental disaster, one study following an oil tanker spill highlighted the main reasons for non-participation to be attitudinal: non-participants had fewer concerns about their own health, or were either not interested in the study or did not think it was useful. While some have provided evidence to the contrary, it is encouraging that some large studies support our finding that a measure of association between an adverse exposure and an adverse health outcome is not appreciably influenced by the inclusion of late participants, as in our study, or the omission of a relatively small proportion of non-participants.

In conclusion, current participants of the Walkerton Health Study appear representative of the general population of interest, and include most individuals who were acutely ill during the outbreak. Our study sample should provide unbiased estimates of the risk of long-term disease after acute bacterial gastroenteritis. Ongoing and future epidemiological analyses will provide a unique opportunity to better charac-
terize these associations following this unfortunate catastrophe.

REFERENCES

12. Accepted: May 27, 2004

RÉSUMÉ

Contexte : Après la contamination bactérienne du réseau municipal d’alimentation en eau de la collectivité rurale de Walkerton, en Ontario, plus de 2 300 cas de gastro-entérite aiguë ont été documentés. L’étude sur la santé de Walkerton évalue actuellement les séquelles à long terme de cette flambée épidi- miologique sur la santé des habitantes et des habitants de Walkerton ayant accepté de participer. Nous avons cherché à déterminer si l’association entre l’exposition aiguë et les premiers résultats sanitaires à long terme pouvait comporter des biais en raison des différences entre les recrues initiales et tardives.


Résultats : Des 4 315 personnes participantes, 2 756 étaient des résidents permanents de Walkerton, soit 55 % de la population de la ville. Sur le plan démographique, l’échantillon était semblable à la population de Walkerton, bien que statistiquement, les femmes aient eu plus tendance à participer que les hommes (l’échantillon comportait 55 % de femmes, alors qu’elles représentaient 52 % de la population, p < 0,01), et que les proportions de jeunes et de personnes âgées aient été plus faibles que prévu (l’échantillon comportait 13 % de personnes de plus de 65 ans, alors qu’elles représentaient 18 % de la population, p < 0,01). Si l’on compare les 3 959 recrues initiales aux 356 personnes recrutées plus tard au prix d’un effort considérable, ces dernières étaient plus susceptibles d’avoir été asymptomatiques durant la flambée (21 % c. 7 %, p < 0,001), mais sur tous les autres plans (âge, sexe, recours aux soins médicaux et état de santé avant la flambée), les deux groupes étaient semblables. Le risque d’hypertension artérielle ou de séquelles rénales à long terme ne différait pas de façon significative chez les recrues initiales et tardives de l’étude.

Conclusions : Les participants et les participants de l’étude sur la santé de Walkerton sont représentatifs de la population de Walkerton, et ils comprennent les personnes atteintes d’infections aiguës durant la flambée d’origine hydrique. L’échantillon disponible pour l’étude devrait donc fournir des estimations raisonnablement imparfaites du risque associé entre la gastro-entérite bactérienne aiguë et les séquelles à long terme sur la santé.