ASSESSING PATHOGENS IN A DRINKING WATER SOURCE:
INVESTIGATIONS IN THE GRAND RIVER WATERSHED

PETER M. HUCK, UNIVERSITY OF WATERLOO

Research conducted 2001-2004; 2005-2007; 2008-2009

Canadian Water Network

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WHY DID WE DO THIS RESEARCH?

Watersheds are an important source of drinking water. Detailed information on the quality of water is crucial for robust treatment plant design to ensure that drinking water systems can effectively remove pathogens at both average and peak concentrations.

The source of pathogens in the watershed can be from either animal or human fecal material. Understanding the origin and relative contributions of fecal loading can be useful for identifying and managing sources of contamination and for identifying and monitoring source water protection initiatives. Because pathogen concentrations can fluctuate considerably in rivers, it is important to obtain information over a long time and through seasonal changes.

Currently, pathogens can be expensive and difficult to measure, although methods to detect various types of pathogens improve over time through research innovations. It is important to use the most up-to-date methods to measure pathogen concentrations to produce accurate results. It is also important to improve pathogen detection methods, since current methods are far from perfect.

Three inter-related research projects were conducted over seven years (2002-2009) in the Grand River watershed, located in southern Ontario. Three treatment plants in the watershed provide drinking water for the Region of Waterloo, City of Brantford and Six Nations of the Grand River. The Grand River is affected by agriculture, treated wastewater and wildlife, and flow and water quality can change rapidly in response to hydrologic events. The urban population in the watershed has increased rapidly in recent years and will continue to expand. Therefore, assessing water quality in the watershed will be important as part of broader source water characterization and protection initiatives.

HOW WAS THE RESEARCH CONDUCTED?

The overall objectives of these three projects were to:

1. Measure baseline and fluctuations in microbial pathogen concentrations in rivers within the Grand River watershed.
2. Assess agricultural and urban sources of pathogens in the watershed.
3. Investigate how watershed seasonal and hydrologic conditions affect pathogen loading.
4. Determine the influence of watershed management incentives in promoting source water protection.
5. Develop new molecular (nucleic acid-based) methods to measure pathogens in drinking water sources.

The studies used improved methods to investigate pathogens in the Grand River watershed. The 2002-2004 study included intensive sampling of all the sub-watersheds and measured pathogen loadings from non-point (agricultural) sources. Later studies in 2005-2009 focused on sample locations within Waterloo Region and included more frequent sampling upstream of a drinking water treatment plant. The sampling in Waterloo Region linked with the Public Health Agency of Canada's (PHAC) C-EnterNet program, which involved surveillance of pathogens from water, retail and farm samples. The later studies also focused on improving pathogen detection methods.

WHAT WERE THE RESULTS?

Sampling at the Conestogo River

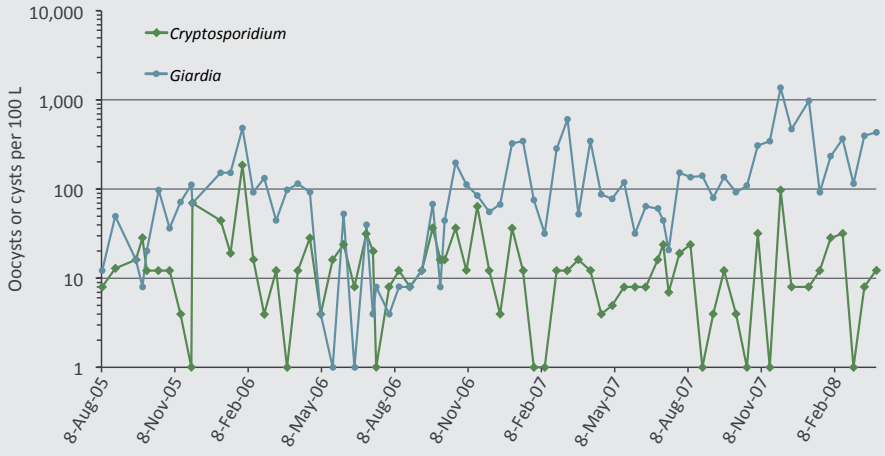
1. PATHOGEN MONITORING

Cryptosporidium and *Giardia* concentrations were measured in the Grand River watershed over a 2 ½ year period. River water was collected every two weeks, primarily in the Grand River near a municipal drinking water treatment plant intake. *Cryptosporidium* was frequently detected in 88% of samples, with a median concentration of 12 oocysts per 100 litres (corresponding to 0.01 oocysts per 100 mL). *Giardia* was detected in 97% of samples at a higher median concentration of 80 cysts per 100 litres (corresponding to 0.08 cysts per 100 mL). Although concentrations of the protozoan pathogens were low, they could vary significantly.

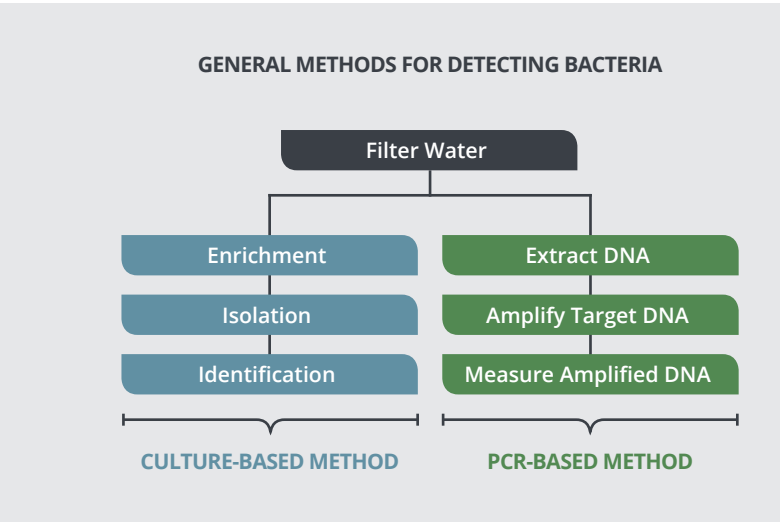
Samples for *Campylobacter*, *Salmonella* and *E. coli* O157:H7 were measured in rivers in the watershed over a three to six year period. Culture-based and PCR (DNA) based methods for pathogen detection were compared, and DNA-based methods detected higher occurrences than culture-based methods. DNA-based methods are more rapid than culture methods and can result in better detection levels and specificity. However, culturing methods can also be useful in studying pathogens because they involve growth of bacteria in the laboratory, and these cultures can then be studied further. *Campylobacter* was frequently detected (70% of samples were positive), but at low concentrations (6 cells per 100 mL on average). *Salmonella* and *E. coli* O157:H7 were rarely detected, and again at low concentrations.

Human enteric viruses in the Grand River watershed (2002-2003) were present in 26% of samples overall, with low concentrations (maximum of 6 infectious units per L). These projects provided important data on pathogens in drinking water sources that were not previously available in this watershed. Results showed that pathogen concentrations in the Grand River were similar to other agricultural and urban impacted watersheds in Canada and worldwide. The data confirmed that federal and provincial drinking water regulations and guidelines for pathogen removal from surface waters ensure the protection of public health.

The pathogen data from these projects were used in subsequent risk assessment studies to calculate the human health risk of pathogens through water exposure pathways. Risk assessment studies can identify and prioritize critical points for pathogen removal and guide regulations. **Studies done for two communities in the watershed verified that the drinking water treatment plants were robust and resulted in excellent protection of public health.**



Protozoan concentrations in the Grand River.



Canagagigue Creek

RELATIONSHIP WITH WATER QUALITY INDICATORS AND HYDROLOGICAL EVENTS

Total *E. coli* levels (non-pathogenic) are a useful fecal indicator that pathogens may be present, but in reality are seldom found to correlate with pathogen concentrations. Weather events can result in increased fecal loading from point and non-point sources, but these events also rarely correlate with pathogen levels.

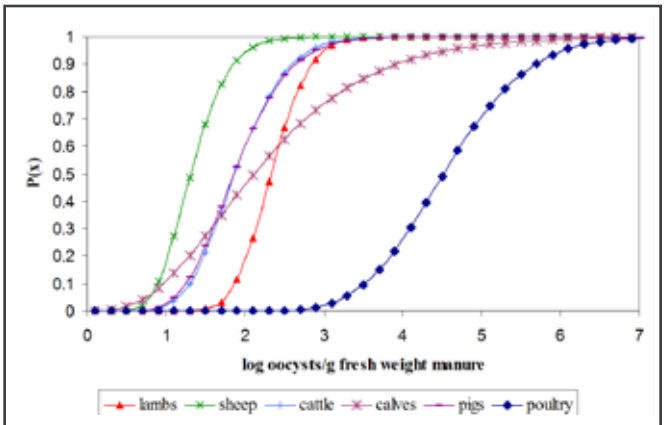
Project results showed that for the pathogens monitored, only *Cryptosporidium* was significantly correlated with *E. coli*, turbidity and river flow. There was no correlation between *Cryptosporidium* and *Giardia*. *Giardia* and *Campylobacter* showed a seasonal trend, with higher concentrations at cold temperatures. Although *Campylobacter* was frequently detected, it was not strongly correlated with any water quality parameter.

E. coli indicators were significantly correlated with turbidity, and rapid increases in *E. coli* concentrations during storm events suggested that bacterial resuspension may be of equal or greater importance than land-based inputs. Research also found that during storm event monitoring, peaks in *Campylobacter* levels did not match peaks in water quality indicators. These results suggest that **water quality indicators are not reliable predictors of pathogen levels**, but remain useful for monitoring source and treatment process performance.

2. IDENTIFYING PATHOGEN SOURCES

Agriculture can be an important source of pathogens, but it can be difficult to measure how non-point sources, including livestock, can affect water quality. To address this issue, the researchers applied models to estimate fecal and pathogen shedding from livestock in the Grand River watershed. Farm census data were used to determine livestock numbers in the watershed, and together with published studies, the number of *Cryptosporidium* and *Campylobacter* produced per year were predicted.

Results showed that although cattle produce the most manure, other farm animals are also important contributors to pathogen loading. Interestingly, *Campylobacter* production was estimated to be higher than *Cryptosporidium* by several orders of magnitude, which corresponded with the watershed pathogen data. *Campylobacter* are known to be a common cause of waterborne disease, particularly in untreated water as they are sensitive to disinfection. The model predicted that the upper agricultural regions of the watershed would have the highest pathogen production. The maximum estimated production of *Cryptosporidium* and *Campylobacter* were located in areas with high levels of clay till and tile drainage, both of which could contribute to increased pathogen loading to surface waters.



Reprinted (adapted) with permission from Dorner, S.M., Huck, P.M and Slawson, R.M. 2004. Estimating potential environmental loadings of Cryptosporidium spp. and Campylobacter spp. from livestock in the Grand River watershed, Ontario, Canada. Environmental Science and Technology, 38(12):3370-3380 . Copyright 2004 American Chemical Society.

Wildlife can also be a source of pathogens in the watershed. *Campylobacter* and *Salmonella* were detected in fecal samples collected from wild birds such as ducks, geese and seagulls. This means that pathogens can potentially be present in water that is not directly influenced by agricultural activities and wastewater. Genotyping analysis, which uses DNA sequence data to compare strains from the watershed with those isolated from animals or humans, demonstrated that farm animals and wildlife were important contributors of *Cryptosporidium* oocysts in the watershed. Human-specific strains were detected in the Grand River, and strains/genotypes of medium to high risk for human infection (*C. hominis, C. parvum and C. ubiquitum*) were in 16% of samples. Therefore, removal of protozoan pathogens is of critical concern for drinking water treatment.

3. PATHOGEN TRANSPORT IN THE WATERSHED

To further predict the sources and transport pathways of microbial indicators and pathogens in the Grand River watershed, a hydrologic model (WATFLOOD/SPL) was modified for pathogen transport and tested using data from a sub-watershed with high agricultural activity. The model predicted that **most microorganisms from land sources enter rivers through tile drainage systems rather than overland transport**. However, the model also predicted that when overland transport did occur, it would result in the highest predicted and observed inputs of microorganisms. This emphasizes that engineering design and risk management plans should pay particular attention to conditions that are difficult to control or predict such as weather-related events.

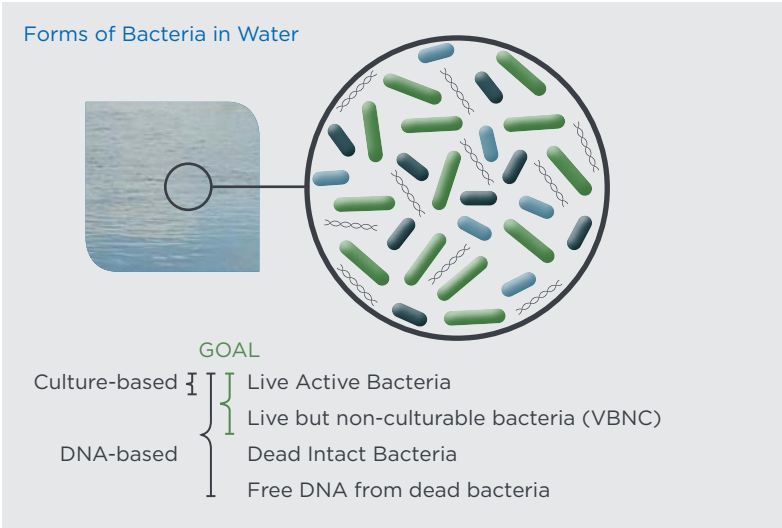
4. EVALUATION OF SOURCE PROTECTION INITIATIVES

In 1998, the Grand River Conservation Authority's Rural Water Quality Program was introduced. Project results showed that financial incentives provided by the program influenced a farmers' decision to participate in watershed protection measures (e.g. improvements to waste management/containment). It was also found that these incentives were positively influenced by the value of the grant, combined with performance incentives.

5. DEVELOPMENT OF PATHOGEN DETECTION METHODS

Researchers examined potential limitations that exist with current pathogen detection methods and developed more rapid and sensitive procedures for pathogen surveillance. These methods can better measure and identify human-infectious strains, and are needed for outbreak investigations, to identify contamination sources, and to guide water practices and regulations.

- An electronic microarray (biochip) technique to detect *Campylobacter*, pathogenic *E. coli*, *Salmonella* and *Vibrio* was developed, which can rapidly identify pathogens from multiple samples at the same time and has both environmental and clinical applications. This method was found to be specific and reliable, and could target living cells that are known to cause human disease. The chip can be reused multiple times.
- A modified PCR (DNA-based) method that could measure living cells was improved, as it is thought that dead cells may cause pathogens to be overestimated using some methods. However, results for several types of bacterial pathogens showed that dead cells were rarely present in the Grand River.
- Improved methods for characterizing emerging pathogens in the watershed found that human infectious strains of *Yersinia* were rarely detected, but *Arcobacter* (a bacteria related to *Campylobacter*) were detected frequently. Further investigation on the importance of these strains in waterborne disease is warranted.
- Researchers developed methods for sub-typing *Campylobacter* strains, which are important for comparing and identifying strains from different sources (human, animal, water). These methods can be used to determine if certain strains can infect humans.



FOODNET CANADA

Pathogen data from this project supported the PHAC C-EnterNet program, now referred to as FoodNet Canada. PHAC was a project partner from 2005-2009. FoodNet Canada supports activities to reduce the burden of enteric disease in Canada, and Waterloo Region was the first sentinel site. This program evaluates how different sources contribute to gastrointestinal disease in humans, to better inform food and water safety policies and evaluate the performance of these policies.

Partnership of the PHAC in this CWN project allowed expanded surveillance of pathogens in water. Project collaboration provided additional resources for more frequently sampling, testing for a wider range of pathogens, and additional characterization data (e.g. PCR detection, genotyping, sub-typing). FoodNet Canada used this data to compare pathogens from various exposure routes, including water, food and animals. The PHAC also worked with public health units to conduct enhanced data collection for human cases in the community.

The FoodNet Canada program has continued pathogen surveillance in the Grand River watershed beyond the CWN project, and has found similar trends over time. The program has evaluated cryptosporidiosis risk factors, and found that recreational water exposure and international travel are important sources of infection. Considering all exposure routes, FoodNet Canada found that *Campylobacter* was the most common cause of enteric disease in the Region followed by *Salmonella*, and for both the most likely source was food (chicken).

The goal of the PHAC surveillance work is to provide practical information on the causes of enteric disease. This is needed to support and guide resource allocation, operations, training and outreach programs for food, agriculture and water industries. The FoodNet Canada surveillance program continues in Waterloo Region, and has since expanded into British Columbia and Alberta. Further information is available at *http://www.phac-aspc.gc.ca/foodnetcanada/index-eng.php*

WHAT ARE THE IMPLICATIONS FOR STAKEHOLDERS AND DECISION MAKERS?

This research characterized the human health implications of a watershed that is an important source of drinking water. Overall results showed that pathogen removal is of critical concern for drinking water treatment.

Pathogens of importance in human disease, including *Campylobacter*, *Cryptosporidium* and *Giardia*, were frequently detected in the watershed. In fact, *Campylobacter* inputs and concentrations in water were several orders of magnitude higher than the protozoan pathogens.

Methods to improve pathogen detection in water were developed and used to identify pathogen types and sources. For example, *Cryptosporidium* strains/genotypes of high risk for human infection were detected in the Grand River, and humans, farm animals and wildlife are all potential contributors of pathogens in the watershed.

Although pathogens were generally detected at low concentrations, levels could fluctuate considerably. Pathogen concentrations were rarely correlated with water quality indicator levels, although indicators remain important for monitoring and treatment performance. Pathogen transport modeling showed that engineering design and risk management plans should pay particular attention to conditions that are difficult to control or predict such as weather-related events.

Project results have been used to support risk assessments to ensure the effectiveness of treatment plant design. Results have also been used to provide a comparative assessment with other pathogen exposure routes including food, and with other watersheds in Canada and worldwide. As well, project findings can contribute to source protection initiatives.

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ONTARIO MINISTRY OF THE ENVIRONMENT
GRAND RIVER CONSERVATION AUTHORITY
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CITY OF BRANTFORD (ENVIRONMENTAL SERVICES)

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