Proposal: The prevalence of STEC and *stx*$^{+}$ wells and microbial source tracking of fecal contamination in rural southern Ontario drinking water wells

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Background**:** Water is the greatest vector of infectious diseases; waterborne pathogens are responsible for multiple diseases including those that are acute, chronic or fatal (1). In the United States, numerous significant infectious disease outbreaks, linked to the contamination of drinking water, are documented each year, (2) and it is thought that most outbreaks go unreported. In Canada, the most notable outbreak of *Escherichia coli* O157:H7 contamination in a municipal water supply occurred in Walkerton, Ontario, and resulted in an estimated 2000 cases, and 7 deaths (1). However, multiple, but minor, 'Walkertons' linked to private well water consumption, likely occur frequently in Ontario, but given these impact single households, or very small populations, they often go unnoted or unrecognized. Therefore, a neglected population is left vulnerable because of lack of attention to this issue. Furthermore, in Ontario specifically, monitoring of private wells for pollutants remains the responsibility of the owner (1). Moreover, potential pathogens are reintroduced into the watershed by those infected, including those that are antibiotic resistant, possibly contaminating more broadly, including the contamination of waterways, livestock and agricultural products (produce) consumed by others. In other words, the problem is epidemic, not recognized, and overdue for study.

Private unregulated wells are the primary drinking water source for 98% of rural Ontarians. Using historical data and Geographical Information Systems (GIS) analyses, research at our laboratory has identified three regions of increased relative risk for *E. coli* contamination ("hotspots") in private wells in southern Ontario (Kingston & Belleville, Niagara, and the Bruce Peninsula – Figure 1) (3). Additional study revealed that there is a cluster of human-sourced fecal contamination in the Kingston & Belleville region (4). Further research is required to develop an understanding of the pathogens present, and their sources, so as to qualify and quantify the human health risk and enable proactive and effective public health and clinical interventions. Moreover, as cattle are a major reservoir of pathogens, notably *E. coli* O157, prior research has identified a region within Ontario, with the highest density of cattle (Figure 2) where residents may also be at elevated risk for pathogen contamination of wells.

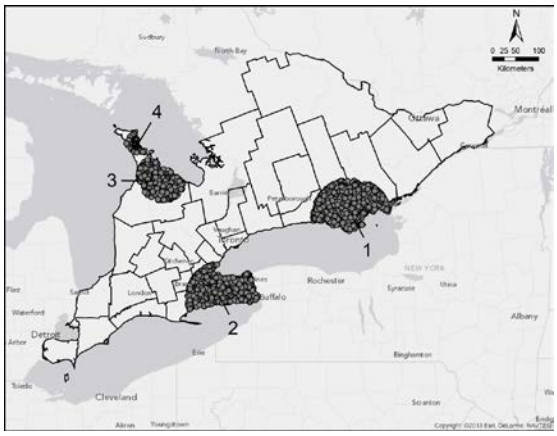

Fig 1: 4 hotspots of E. coli contamination among private well water samples submitted for testing to PHO in 2012

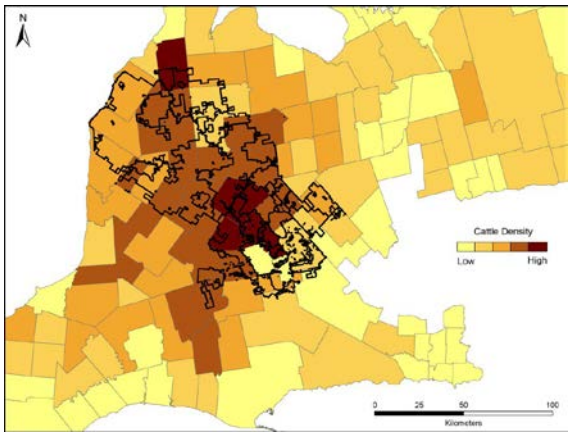

Fig 2: A map of cattle density with a postal code catchment boundary overlay

Historically, enteric pathogens have been isolated from drinking water wells, either as a result of outbreak investigations or scientific studies. Several outbreaks have been linked to *Campylobacter jejuni* (5) including a 1985 one in Orangeville, Ontario where groundwater was contaminated with agricultural runoff. Septic tanks were suspected to be the source of *Shigella sonnei* in two US outbreaks (in Florida and Idaho) (5). *Yersinia* was isolated in 16% of rural wells during a Finnish study (6). Given the cases of pathogen presence in wells causing human illness, especially in Ontario, investigating their prevalence in regions with high fecal contamination of wells or high density of cattle is essential to determine the level of risk to rural populations.

Objectives: During summer, 2014, regional research collaboration between the Kingston, Hamilton and London Public Health Ontario Laboratories (the laboratories which capture the four regions of concern) will begin. The project aims to determine the fecal source of private well contamination using MST and the prevalence of STEC and *stx*$^{+}$ organisms in wells shown to be contaminated with bovine feces. Additionally, the project aims to determine the prevalence of enteric pathogens in the collected *E. coli* positive samples. This will aid in answering two research questions:

1. What is the prevalence of *Shigella, Campylobacter, Yersinia, Salmonella, E. coli* O157 and STEC in *E. coli* positive private drinking water samples, in the four regions of concern?

2. Is there a difference in the prevalence of each pathogen among the four areas? And, if so, is the difference related to land cover, land use, socio-economic status or hydrogeology and what does it mean with respect to area specific interventions and human health risks?

All of this information will be used to support the risk analysis tool currently being developed for PHUs and private drinking well owners, in order to better understand the needs for testing and intervention for their particular drinking water sources and regions.

Research Plan:

1. **Geographic Locations**: The regions for sample collection are based upon previously identified areas of increased relative risk for *E. coli* contamination.
2. **Sample Size**: Sample size is based upon historic data retrieved from the Water Testing Information System (WTIS), and an average annual *E. coli* positivity rate of between 4 and 7%.
3. **Sample processing**: Participating laboratories will receive and process all water samples, by filtering 100 ml of water and membrane filtration, as per standard protocols,, and cultured for *E. coli* and total coliforms. Between 100-300 *E. coli* + plates/samples will be shipped to the PHOL-Kingston for further assessment. The remaining 100 ml of the initial 200 ml provided will also be filtered and DNA extracted at the receiving laboratory. Samples will be shipped to Kingston the day read, or the day following, by the primary laboratory. Samples must be kept at 4^{o}C. Samples must be clearly identifiable with respect to bar code, and a copy of the requisition with the results will accompany each specimen.
4. **Pathogen culture detection**: Filters will be re-suspended in non-selective broth, incubated overnight and sub-cultured to enteric media specific to the detection of the standard pathogens currently employed in routine clinical laboratories; namely, *Shigella, Salmonella, Yersinia, Campylobacter and E. coli* 0157:H7. A non-differential, non-selective agar will also be added to the series. Further, sorbitol fermenting *E. coli* colonies will be sub-cultured to Colorex STEC agar, which has been previously determined as capable of detecting other STEC strains, including O26, O45, O103, O111, O121, and O145. (Linda Chui, personal communication). As a summer student, I will fulfill this particular role.
5. Analysis: The results of each test will be mapped spatially and statistically compared to the land cover, land use, hydrogeology and socioeconomic status within the regions of investigation.

References

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