The prevalence of STEC and *stx*+ wells and microbial source tracking of fecal contamination in rural southern Ontario drinking water wells

Applicant: Sophie Felleiter, Year 4, Life Sciences Specializations, Queen's University
Supervisors: Dr. Anna Majury, Clinical Microbiology, Public Health Ontario, and Dr. Gerald Evans, Department of Medicine, Queen's University

The objective of the project for the period of the Summer 2014 was to identify the prevalence of standard pathogens of concern including *Shigella*, *Campylobacter*, *Yersinia*, *Salmonella*, *E. coli* O157, and STEC in *E. coli* positive drinking water samples in four regions of concern previously identified[1]. These hotspots included Kingston/Belleville, captured by Public Health Ontario Laboratory (PHOL)-Kingston, the Niagara region, captured by PHOL-Hamilton, and the Bruce Peninsula captured by PHOL-London. In addition, the London and Hamilton laboratories captured samples from a high cattle density area within southern Ontario where drinking water wells may also be at a risk of contamination.

Participating laboratories received and processed all water samples by filtering 100 ml of water as per standard protocols, and cultured for *E. coli* and total coliforms. 577 samples in total were received and processed between the dates of June 2nd-August 29th. Total *E. coli* numbers were less than expected in the first half of the study (May/June): therefore total coliform (TC) positive plates were included in the study to ensure sample size was adequate. Sample details can be found in Table 1. The remaining 100 ml, of the initial 200 ml provided, was filtered and DNA extracted at the receiving laboratory. Samples were shipped to PHOL-K the day read, or the day following, by the primary laboratory. Samples were kept at 4^{o}C prior to processing.

Table 1: Breakdown of the total samples received and processed by the host laboratory

Location	**Total Coliform + (TC's)**	***E. coli'* +**	**Total**
Bruce Peninsula	49	132	184
Niagara	72	93	165
Kingston/Belleville	26	30	56
Cattle Dense	103	69	172
			577 total samples

Upon arrival at PHOL-K, filters were re-suspended in peptone and sub-cultured to enteric media. Specifically, Maconkey, Sorbital Maconkey, Hektoen, CIN, and CSM, which are specific to the detection of the standard pathogens currently employed in routine clinical laboratories. Selenite broth was used in an attempt to isolate *Salmonella spp.* A non-differential, non-selective agar was also added to the series (Columbian Blood Agar) for the purpose of streaking the first quadrant of the grown plate, beading and then freezing at -80^{o}C for storage and future analyses. Further, Colorex STEC agar was used to identify not only O157 but also has been previously determined as capable of detecting other STEC strains, including O26, O45, O103, O111, O121, and O145.

After initial screening through enteric media, samples that displayed colonies typical of the standard pathogens underwent further testing via various biochemical agar tubes. This included a Triple Sugar Iron agar for the differentiation based on the fermentation of three sugars and H_2S production, an ONPG-PA-M sulphate medium for the detection of motility,

Galactosidase activity, phenylalanine deaminase activity and H_2S production, and a Urea agar for the detection of rapid urease activity. These tests were used in the case of suspect *Shigella, Salmonella, E. coli* O157 and *Yersinia* colonies. Suspect *Shigella, Salmonella* and *Yersinia* via slants underwent biochemical analyses using API 20E to determine the organism in question. Suspect *E. coli* from the slants underwent O157 latex test for confirmation. If a colony was determined by the API to be a pathogen of interest, it was beaded and frozen at -80^{o}C for future analyses.

In the case of *Campylobacter*, colonies that grew on the CSM plate were tested with oxidase solution. If oxidase positive, colonies underwent a gram stain to determine whether the characteristic seagull of the *Campylobacter* genus. Suspect colonies were beaded and frozen at -80^{o}C for future analyses.

Of the 577 samples total received, 19 had significant evidence of contamination of a standard pathogen as seen in Table 2. It was found that Non-Lactose fermenters on Maconkey agar, Non-sorbitol fermenters on Sorbitol Maconkey (suspected O157) agar and green colonies on Hektoen agar (suspected *Shigella*) were mostly found to be other organisms some of the more common including *Serratia spp, Pantoea spp, Enterobacter spp,* and *Non-fermenter spp*. Further, the majority of black colonies on Hektoen agar (suspected *Salmonella*) were most often *Citrobacter spp* or *Hafnia spp,* which is expected because their morphology on culture is similar. Many of these other organisms discovered are typically not pathogenic and are found within the human gut flora. No *E.coli* O157 were identified via latex tests and no organisms were determined to be Campylobacter through gram stains. Lack of *Campylobacter* via culture is expected due to its ability to pass through standard filters easily[2] and general fragility of the species[3], particularly when attempting to isolate from environmental waters.

Table 2: Breakdown of the total pathogens found from all samples processed

Location	***Salmonella spp***	***Shigella spp***	***Yersinia spp***	**Total**
Bruce Peninsula	5	2	0	7
Niagara	2	2	0	4
Kingston/Belleville	1	0	0	1
Cattle Dense	4	0	3	7
				Total 19 Pathogens

There are a number of possibilities why few pathogens of interest were isolated from these locations. It is possible that pathogens of interest are present, but perhaps in a viable but non culturable state (VBNC), meaning that they cannot be cultured successfully using traditional methods but could still detect them through more sensitive techniques such as real-time PCR (rt-PCR. This is a promising explanation as it has been shown numerous times for a variety of organisms that rt-PCR are much more sensitive and efficient than traditional culture methods[3,4]. It is also possible that the pathogens of interest are being outcompeted by the *E. coli* and coliforms that are present in water sample.

After DNA extraction of all samples, real-time PCR will be conducted in order to detect pathogens of interest using a more sensitive method. This laboratory has rt PCR assays, previously validated for drinking well water samples, available for *Campylobacter jejuni*, *stx* 1/2 assay, and *E. coli* , A *Salmonella spp*. rt-PCR assay for use with drinking well water samples,is currently under development. Molecular Microbial Source Tracking (MST) assays for the

detection of the fecal source of contamination will also be employed using these samples. Once this information is gathered it can be mapped spatially and statistically compared to the land cover, land use, hydrogeology and socioeconomic status within the regions of investigation.

References

1. Krolik J, Maier A, Evans G, Belanger P, Hall G, Joyce A, Majury A. (2013) A spatial analysis of private well water Escherichia coli contamination in southern Ontario. Geospatial Health 8(1):65-75.
2. Donnison, A. (2003) Isolation of Thermotolerant *Campylobacter*- Review & Methods for New Zealand Laboratories. Ministry of Health, New Zealand.
3. Yang, C., *et al*. (2003) Application of real-time PCR for quantitative detection of *Campylobacter jejuni* in poultry, milk and environmental water. *Immunol. Med. Microbial.* 38, 265-71.
4. Moore, J., *et al*. (2001) Molecular Detection of *Campylobacter* spp. In drinking, recreational and environmental supplies. *Int. J. Hyg. Environ.* Health. 204,185-9