ALBERTA INNOVATES

CLEAN ENERGY FINAL PUBLIC REPORT

1. PROJECT INFORMATION:

Project Title:	**Assessing Water Quality, Microbial Risks and Waterborne Pathogens in Rural Alberta using a One Health Framework**
Alberta Innovates Project Number:	AI 2199
Submission Date:	June 30th 2020
Total Project Cost:	$1,206,430
Alberta Innovates Funding:	$466,430
AI Project Advisor:	Susan Carlisle

2. APPLICANT INFORMATION:

Applicant (Organization):	**University of Calgary**
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3. PROJECT PARTNERS

Please provide an acknowledgement statement for project partners, if appropriate.

RESPOND BELOW

We would like to acknowledge Alberta Agriculture and Forestry, Alberta Health Services, the Public Health Agency of Canada and Alberta Precision Laboratories for their support of the project. We also acknowledge numerous student funding partners.

A. EXECUTIVE SUMMARY

Provide a high-level description of the project, including the objective, key results, learnings, outcomes and benefits.

RESPOND BELOW

This research project focuses on water quality protection. This project is co-funded by Alberta Agriculture and Forestry. Researchers aim to identify and characterize factors relating to groundwater contamination in Alberta through use of geographic information system (GIS) mapping and spatial analysis to assess microbial risks associated with Shiga toxin-producing *Escherichia coli* (STEC), antimicrobial resistance (AMR), and viruses. The results will be compared with work previously and concurrently carried out using the current drinking water quality guidelines (total coliforms and *E. coli*) in Canada. The project also aims to better understand livestock producers' perceptions of the source of and importance of well water contamination to determine how this affects their livestock and well management decisions.

Objectives are:

1. To perform a retrospective survey of archived *E. coli* positive samples from well water submissions to a provincial laboratory to detect whether these *E. coli* are resistant to antimicrobials and whether they are STEC.
2. To prospectively sample well water within a sentinel region for the presence of bacteria and viruses.
3. To describe the patterns of STEC and antimicrobial resistant organisms in well water.
4. To perform source tracking of faecal contamination in *E. coli* positive wells.
5. To examine well owners' perceptions of water quality and contamination and the influence of their perceptions on their management practices.
6. To provide information to decision makers on implications for human, animal and environmental health.

One of the key outcomes of this project is the creation of the transdisciplinary research group who used a One Health approach to interpret and apply results for the benefit of human, animal and environmental health. Overall, this project, thus far, has 6 peer-reviewed publications, 10 draft publications, 5 lay articles,

3 graduate theses, 1 undergraduate honours thesis, 1 co-op student thesis, 10 oral conference presentations, and 13 poster conference presentations. In addition, 9 jobs were created during the project, 11 students were trained, and 3 new products / services were created (One Health approach; a technique for source tracking water contamination and molecular water quality assessment; water sampling device for virology testing).

The knowledge generated by this project will result in:

- Better understanding of the potential risks related to well water confirmed to contain faecal contamination.
- Identification of wells that are consistently compromised when current water testing standards fail to detect that contamination, leading to improvements to standard methods used to identify well water contamination.
- Refinement of a well vulnerability risk assessment tool used by the health authorities to make assessments based on the physical characteristics of the well.
- Development of new policy guidelines to promote uptake of free well water testing in the province.
- Partnership between researchers and the Alberta Health Services to encourage best practices for water well management.

INTRODUCTION

Please provide a narrative introducing the project using the following sub-headings.

- **Sector introduction:** Include a high-level discussion of the sector or area that the project contributes to and provide any relevant background information or context for the project.
- **Knowledge or Technology Gaps:** Explain the knowledge or technology gap that is being addressed along with the context and scope of the technical problem.

RESPOND BELOW

Sector introduction

Microbiological quality of Alberta source water for drinking:

Alberta's rural population is estimated at 450,000 – 600,000 people all of whom depend on private groundwater or stored water systems (i.e., cisterns) as a source for drinking water (Summers 2010). Studies have demonstrated that as many as 25% of these water systems fail current water quality parameters for potable water, as outlined in the Guidelines for Canadian Drinking Water Quality (Health Canada, 2012). Although the guidelines recommend that private systems should be tested 2 - 4 times per year for microbial water quality, it is estimated that only 60% of Albertans will test their water even once over a five year period, leaving 40% of the population consuming water of unknown quality. Regulations require that drinking water be tested for total coliforms and *E. coli*. Any total coliform or *E. coli* positive well is considered abnormal and is brought to the attention of public health authorities for action.

Studies have demonstrated that consumption of untreated or improperly treated groundwater can be associated with the transmission of waterborne pathogens (Alary and Nadeau, 1990; Beller et al, 1997,

Bruce-Owen-Sound, 2000, Millson et al, 1991). Outbreaks in the last couple of years, with *E. coli* O111 in 2008, O145 in 2011 causing complications such as haemolytic uremic syndrome and deaths, clearly demonstrate the virulence of these organisms. The utilization of untreated ground water can also be a potential threat to food safety where it is used for irrigation (especially of salad crops and fruits/vegetables that are not well washed).

Knowledge or Technology Gaps

Through previous research funding (Water for Life, Alberta Water Research Institute [Safe, Secure Water for Alberta]), and in conjunction with Alberta Health and Alberta Health Services and the Provincial Laboratory for Public Health (ProvLab), now Alberta Precision Laboratories (APL) (Checkley, Neumann, Valeo), we have used geographical information systems (GIS) to spatially and temporally map rural water quality in Alberta. APL is responsible for testing upwards of 250,000 water samples/year of which ~10,000 – 15,000 originate from private well water systems, and represents a rich source of data for surveillance of rural water quality in Alberta. Our spatial analysis of rural groundwater quality systems revealed areas of concern in Alberta, as determined by a higher than normal proportion of groundwater wells failing microbiological potable water quality guidelines within distinct geographical areas of the province. Moreover, geotemporal variation (i.e., seasonal and climatic) in microbiological water quality was also observed in certain areas of the province, suggesting that infusion of surface water into groundwater systems is affected by seasonal/climatic variables. Our team's previous complementary research (i.e., GIS and epidemiological studies) has demonstrated that both human (i.e., age of septic system) and animal sources (i.e., livestock) are significant risk factors associated with contamination of rural groundwater supplies. The goal of this research is to further investigate these associations to better understand risks to human and animal health.

Presence of antimicrobial-resistant E. coli in source water

Our internal research has demonstrated that more than 16% of *E. coli* contaminated wells in Alberta were shown to carry antimicrobial-resistant *E. coli*, many of which were multi-drug resistant. Many of these were found to be resistant to four major classes of antimicrobials, with resistance to upwards of 10 different antimicrobials. Consumption of groundwater might be associated with an increased risk of carriage of antimicrobial resistant *E. coli* in people. The problem of antimicrobial resistance is likely to be far more problematic considering the total coliform group of bacteria: total coliform failures in rural drinking water systems exceed *E. coli* contamination by a ratio of 10:1 in Alberta, and yet the rates of antimicrobial resistance in these multiple genera in Alberta's groundwater systems are largely unknown.

Risk management for human and animal health:

Source water contamination by bacteria and viruses can be significant risk factors to both human and livestock animal health. We need to better understand livestock producers' perceptions of the source of/importance of well water contamination, different levels of contamination, and contamination with different pathogens so that we can determine how this affects their management decisions. A prototype near real-time temporospatial surveillance system has been set up by our team for monitoring drinking water throughout Alberta using water quality indicators. We are analyzing the association of water quality indicators with animal distribution and animal disease data as well as human laboratory confirmed disease data. The project will provide scientific evidences to support authorities to develop or revise the water policy to mitigate the risks.

Systemic information linking to development of risk management plan:

Although some of previous studies provided evidence of microbiological quality of source water for drinking in rural well water in Alberta, a systemic temporospatial analysis and information package are still missing. A detailed evaluation is required to characterize the presence and seasonality of waterborne pathogens in rural groundwater, and their relationship with hydro-geological pattern and association with disease outbreaks. This will help better understand the environmental role in waterborne pathogen transmission. Robust pathogen risk maps leading to more comprehensive understanding of the problems is essential for comprehensive risk mitigation strategies. This will ultimately enhance competitiveness and profitability of the industry. The systemic information package will lead to a clear pathway to improve on-farm biosecurity and water management as well as help Alberta livestock producers and processers in a practical way.

Human and animal viruses in rural well water system and its associated risk:
There is a lack of information on the occurrence and characterization (e.g. well depth/location etc.) of human enteric viruses in rural groundwater used for private drinking water. These are required inputs to better understand health risks associated with these rural private drinking water systems. Enteric viruses are extremely contagious due to their low infectious dose and high level of shedding. They also have diverse transmission routes (foodborne, waterborne) and can persist in the environment for a long time. Our studies have concluded that human enteric viruses are responsible for 80% of gastroenteritis outbreaks in Alberta (Pang et al. 2010). Other studies reported up to 38% of illnesses related to drinking water contamination are associated with viruses (Blackburn et al. 2004). Monitoring *E. coli* and fecal coliforms in source and drinking water based on current microbiological standards does not provide a reliable assessment of risks related to viral pathogens in the water systems. We need to further investigate this relationship. This problem has been identified by the guideline for Canadian Drinking Water Quality and the Alberta groundwater quality assessment. Moreover, there is a lack of evidence-based information regarding the prevalence of enteric viruses in Alberta's groundwater, especially in rural water systems. Two major technical hurdles are 1) valid and reliable methods to detect viruses in various water systems, and 2) epidemiological analysis to better understand the relationship between the levels of viral contamination in source and drinking water and disease burden. Obviously, insufficient data on microbiological contaminants (bacteria and viruses) in groundwater and drinking water makes it difficult to establish guidelines, evaluate the vulnerability and ensure safety of source water for drinking. In our series of studies on human viruses in wastewater and surface water, abundant viruses were present in post-treatment discharges of wastewater and downstream sites of municipal wastewater treatment plants (WWTPs) of river water in 6 major rivers across Alberta (Qiu et al. 2018, Pang et al. 2019). With our validated technology/experiences on detection of enteric viruses in various water matrices and the innovative sampling device for large volumes of water, it is time to fill the knowledge gap on presence and levels of enteric viruses in Alberta groundwater and to understand a whole spectrum of microbiology quality of groundwater in Alberta for rural development and environmental stewardship.

B. PROJECT DESCRIPTION

Please provide a narrative describing the project using the following sub-headings.

- **Knowledge or Technology Description:** Include a discussion of the project objectives.
- **Updates to Project Objectives:** Describe any changes that have occurred compared to the original objectives of the project.
- **Performance Metrics:** Discuss the project specific metrics that will be used to measure the success of the project.

RESPOND BELOW

This collaborative project was designed to provide a comprehensive assessment of water quality, microbial risks and waterborne pathogens in rural Alberta using a One Health approach. As such the overall project involved a number of sub-projects that were addressed under a number of different objectives. The objectives of the project and the tasks set within each objective are outlined below. For the purpose of this report the sub-projects will be referred to as the following; STEC study (objectives 1,2,3), AMR study (objectives 1,2,3), recruitment study (objective 2), virology study (objective 2), extended pathogen screening and contamination persistence study (objective 2), source tracking study (objective 4), perceptions study (objective 5), and a vulnerability mapping study, a VRAT study, faucet study, creation of a Tableau interface and a rare event data study (objective 6).

Objective 1: Perform a five year retrospective survey (2008-2012) of 1200 ProvLab archived *E. coli* isolated from positive well water samples from across Alberta for Shiga toxin-producing *E. coli (STEC)* and antimicrobial resistance (AMR) in *E. coli*.

- Task 1.1. Test all archived *E. coli* positive private well water samples for stx 1 and stx 2 using qPCR on stored repositories.
- Task 1.2. Serotype *E. coli* from STEC positive wells in retrospective survey.
- Task 1.3. Screening for antimicrobial resistant *E. coli* in retrospective study by the agar screen plate method.

Objective 2: Prospectively sample and characterize well water across Alberta and within a rural sentinel area in Alberta with respect to water quality indicators (presence or absence of total coliform (TC), *E. coli* (EC)), STEC, AMR in *E. coli,* enteric viruses and other pathogens as appropriate.

- Task 2.1. Routine prospective testing of wells for *E. coli*/total coliforms and testing *E. coli* positive wells for STEC
- Task 2.2. Collect private well water samples from 90 livestock operations within the sentinel region, chosen based on a sampling frame as part of another One Health surveillance project. Routine tests for water quality indicators.
- Task 2.3. Choose a subset of 50 wells, stratified by depth of well (deep, shallow), to use for virus testing viruses once monthly for 24 months to assess occurrence and seasonality of water contamination. The water quality indicators (TC and EC) will also be performed at each water collection so associations between enteric viruses and water quality indicators can be made seasonally accounting for depth of well.

- Task 2.4. Screening for antimicrobial resistant E. coli in prospective study by the agar screen plate method, and including extended spectrum beta lactamase producing *E. coli*.
- Task 2.5. Identification of repetitive well failures and perform extended pathogen testing (Salmonella, Campylobacter, Cryptosporidium, Giardia)

Objective 3: Describe the temporal and spatial patterns of STEC and antimicrobial resistant organisms in well water across Alberta, both retrospectively and prospectively, and enteric viruses prospectively by assessing associations with environmental (climatic, geologic) and animal husbandry risk factors.

- Task 3.1. Collect GIS information relevant to animal husbandry, land-use demographics, etc.
- Task 3.2. Data integration of GIS information and laboratory water quality and pathogen surveillance

Objective 4: Prospectively source track faecal contamination from *E. coli* positive wells within the sentinel region to assess epidemiological risk factors associated with contamination.

- Task 4.1. Test all *E. coli* positive wells for source of contamination using qPCR for Bacteroides.

Objective 5: Examine livestock producers' perceptions of water quality and contamination and the influence of their perceptions the management practices they choose related to mitigation of water contamination by cattle waste within the sentinel region.

- Task 5.1. Enroll 500 livestock producers in this study
- Task 5.2. Develop and implement questionnaires, focus group discussions, and facility mapping that will be used to investigate perceptions (via probit analysis) and develop qualitative objective assessments relating to water and public health perception, water and manure management biosecurity procedures, and zoonotic disease mitigation strategies.

Objective 6: Use information gained from the study to inform decision makers on the implications for human, animal and environmental health (e.g. water testing policies (microorganisms to test, lack of regulation of testing for private drinking water), risk maps, livestock biosecurity and other mitigation strategies).

- Task 6.1. Provide an overall assessment to policy makers about potential risks associated with private well water systems as well as potential mitigation strategies for human, animal and environmental health.
- Task 6.2. Provide recommendations regarding water testing frequency and microorganisms tested, livestock biosecurity and management and public perception management between agricultural productivity/economy and public health protection.

STEC Study

Knowledge and technology: This study conducted at the University of Alberta utilised previously cryopreserved and archived (retrospective) *E. coli* positive private well water samples (2004-2014), alongside prospective samples (2015-2016) not subjected to the freezing and storing procedure. All samples were analysed by quantitative polymerase chain reaction (qPCR) against the *stx* genes to determine the potential presence or absence of STEC. Isolates were serotyped at the Canadian National Microbiology Laboratory using the QIAxcel® Advanced system. Unique genetic isolates were tested via a Vitek® Automated Bacterial Identification System (bioMerieux, Marcy-l'Étoile, France).

Updates to project objectives: For STEC testing there were 2,042 *E. coli* positive samples analysed dating from 2004 – 2016, including four years of data outside of the original objective and surpassing the objective goal.

Performance metrics:
The success of the STEC study can be measured by 1) laboratory analysis of the samples completed, 2) charts and maps describing patterns of STEC from *E. coli* positive wells across Alberta over time (temporally and spatially) created, 3) presentation of the findings via conferences and publications, 4) providing data that will link to other research ongoing within the sentinel area, 5) training of highly skilled personnel including one MSc student a laboratory technician and a research associate.

AMR Study

Knowledge and technology: This study conducted at the University of Calgary utilised samples as described under the STEC study, cryopreserved and archived (retrospective) *E. coli* positive private well water samples (2006-2014), alongside prospective samples (2015-2016) not subjected to the freezing and storing procedure. The technology used included established methodology for isolation and identification of *E. coli* bacteria. Extended spectrum beta-lactamase producing *E. coli* (ESBL) were identified using Clinical and Laboratory Standards Institute (CLSI) standards. Antimicrobial resistant isolates were identified using the National Antimicrobial Resistance Monitoring System (NARMS) Sensititre™ panel.
Updates to project objectives: The planned analysis was to include 1200 archived samples collected between 2008-2012. Archived samples from 2006 onward were included in this study. The number of samples analysed for the presence of AMR fell slightly short of the goal at 1129 samples because this was the number of samples that were actually archived (there were 1328 samples positive for *E. coli* of which 199 were not archived). For each *E. coli* sample analysed for this project there were approximately 20 presumptive *E. coli* isolates which were characterised, meaning that a total of 20,902 isolates were analysed. This was a very labour-intensive task and surpassed the objective goal. In addition, the methodology was updated to identify ESBL and perform phylogenetic group testing: Isolates flagged as potential ESBL producers that conferred resistance (or were intermediate) to a third-generation cephalosporin, ceftriaxone, via the SWIN® software system (Thermo Fisher Scientific, Burlington, ON, Canada) were flagged. AmpC- and ESBL-producing isolates were distinguished using Mast® 68C ESBL and AmpC detection kit (Alere, Ottawa, ON, Canada) containing four disks containing cefpodoxime (10 µg) in combination with clavulanate (ESBL inhibitor) and/or cloxacillin (AmpC inhibitor) Mueller Hinton agar was inoculated according to the manufacturer's recommendations and the CLSI guidelines. Phylogenetic groups of the 27 potential ESBL-producing E. coli isolates were determined as per the triplex PCR scheme described by Clermont et al. (2000).

Performance metrics:
The success of the AMR study can be measured by: 1) pilot study to solidify methods, and laboratory analysis of the 1129 samples 2) charts and maps describing patterns of AMR resistance from E. coli positive wells across Alberta over time (temporally and spatially), 3) presentation of the findings via conferences and publications, 4) providing data that will link to other research ongoing within the sentinel area, and 5) training of highly skilled personnel include one MSc student and two research assistants.

Recruitment Study

Knowledge and technology: Questionnaires tailored towards three livestock groups (broiler farmers, feedlot owners, and cow/calf producers) were designed and implemented in consultation with the Public Health Agency of Canada's Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and the research team. A fourth questionnaire, similar to the livestock focused versions, but excluding questions relating to production and antimicrobial use, was also implemented.

Updates to project objectives: The initial plan was to recruit 90 livestock operations from which a questionnaire would be administered, and water samples would be taken once per year for three years. It was proposed that the 90 participants would be balanced between broiler farms, feedlots and cow/calf producers. Due to difficulties recruiting participants through local veterinary clinics, the recruitment strategy was expanded to include acreage owners living in close proximity to livestock operations, recruited via advertisements on social media and via water well workshops. A total of 110 participants were enrolled into the study. This included participants that were only enrolled in a well vulnerability risk assessment study (VRAT). Only a small subset of participants provided the once yearly sample over three years.

Results from the 90 farms were to be linked to other animal testing being done on these farms to provide more information within the newly designated sentinel area. This is still occurring using data from this and related projects. Ongoing studies in a subsequent project will meet this particular objective. Farms were enrolled and sampled in our study that were also enrolled into other studies. They had subsequent samples collected from them that are linked to our samples. Ongoing projects assess the genetic relatedness of antimicrobial resistance in *E. coli* from the broilers, cattle and water samples from these farms and model the transfer of antimicrobial resistance across the cattle production chain.

Performance metrics: The success of the recruitment study can be measured by: 1) meeting the recruitment goal and successful collection of a questionnaire and a water sample from each participant 2) analysing the responses to the questionnaire and linking these responses to the water test result for each participant 3) presentation of the findings via conferences and publications.

Virology study

Knowledge and technology: Under the objective 2, human enteric viruses in groundwater were detected prospectively and the results were correlated with the presence of total coliforms (TC), *E. coli* and *Enterococcus* spp. in the household tap water sampled on the same day from the corresponding wells in the selected sentinel region of Alberta and beyond from 2015 to 2018. The key technologies we used were: a) Real-time quantitative PCR panel testing to detect genetic signals of human enteric viruses, including rotavirus, norovirus GI and GII, adenoviruses, enterovirus, astrovirus, sapoviruses, reovirus, and JVC virus; b) viral cell culture and c) integrated cell culture with qPCR (ICC-PCR) for cultivable viruses (rotavirus, enterovirus, adenovirus, reovirus). These assays were developed in Dr. Pang's lab and used in detection of human enteric viruses in various water matrices successfully (Pang et al. 2012). The sensitivity of qPCR method allows for detection and quantification of the levels of viruses in water sample (dynamic range 10 ^1 to 10^7 genetic copies of viruses/liter), providing confidence and solid technical support for pursuing success of this tasks.

Updates to project objectives*:* A subset (50) of the 90 wells stratified by depth of well were to be recruited for virus testing. From these wells samples were to be collected monthly for 24 months. However, due to the labour intensiveness of the sampling procedure and the low number of positive tests a new protocol was put in place whereby all wells were only tested for up to 12 months. Positive wells were to be tested longer based on future results. The goal was accomplished within planned duration and the budget.

Performance metrics: Following metrics are used to measure the success of this task: 1) Groundwater samples meeting quality requirement for the standard microbiology testing for viruses. 2) Comprehensive pre-analytical sample preparation and testing procedures with QA/QC controls for each sample in the lab (totally 578 samples). 3) data updates and summaries for the project progress reports completed on time; 4) communication and meeting presentation; 5) scientific publication (the manuscript is under

preparation with assistance from the Alberta Environment and Parks and intended to submit to Water Research as a series of publications on wastewater, river water and groundwater in Alberta) and 6) training of highly skilled personnel including two summer students, one laboratory technician, a project co-ordinator, and a research associate.

Extended pathogen screening and contamination persistence studies

Knowledge and Technology: Standardised methods of DNA extraction using the EPA Method 1611 were used. All qPCR assays were run on an ABI Fast cycler. Reactions were performed with ABI Fast Advanced Master Mix (ThermoFisher) or PrimeTime Master Mix (Integrated DNA Technologies).
Updates to project objectives: Cryptosporidium and Giardia were not included in the extended screening. (task 2.5). This was because the methodology required to detect these particular pathogens is very different to that used to detect the other markers used in this study
Performance metrics:
The success of the STEC study can be measured by 1) techniques for and summary of all source tracking done on wells within the sentinel region, 2) comparison of this test with standard tests, 3) development of policy recommendations for drinking water testing, 4) presentation of the findings via reports and publications, 5) providing data that will link to other research ongoing within the sentinel area, and 5) training of highly skilled personnel including one postdoctoral fellow, a research associate, a research assistant and a laboratory technician.

Source tracking study
Knowledge and Technology: The knowledge and technology utilized for this sub-project extended the molecular techniques used above and used GIS and statistical methods to analyse persistent contamination in a case series of problem wells.
Updates to project objectives: The component of this study that related to the task set in objective 4 was met.
Performance metrics: The success of the source tracking study can be measured by 1) techniques for and summary of all source tracking done on wells within the sentinel region, 2) development of policy recommendations for drinking water testing, 3) presentation of the findings via reports and publications, 4) providing data that will link to other research ongoing within the sentinel area, and 5) training of highly skilled personnel including one postdoctoral fellow, a research associate, a research assistant and a laboratory technician.

Perceptions study

Knowledge and Technology:
Updates to project objectives: The initial objective was to enroll 500 livestock producers through a combination of the 90 recruited in the sentinel site area and 410 via other methods such as online questionnaires in the same sentinel area. From the recruitment study, 106 samples were utilized. An additional 350 useable questionnaires from respondents enrolled through a combination of mailed and online survey responses were utilized. An additional 200 respondents began but did not finish the questionnaire sufficiently to allow those data to be included in this study. Therefore the target sample size for this objective was not quite met, but was adequate for our purposes. No water samples were taken from sources such as cattle holding facilities; sampling from surface water was taken from sites were possible.

Facility mapping to identify geospatial risk factors associated with manure management and water source was completed on a small subset of wells from the recruitment study as part of the VRAT pilot project.

Performance metrics: This project allowed for the training of one doctoral student, leading to three successful peer reviewed publications. Three summer students were also involved in work on this project.

Objective 6

This objective will be met

Updates to project objectives: There were no updates to the project objectives

Performance metrics Currently there are six publications relating to findings of this project. Additionally, there have been reports prepared relating to the VRAT, rare event study and the faucet study. Two students successfully completed post graduate training programs at masters level. A PhD student partially funded by this project also completed their program. A post-doctoral fellow completed a four year term co-ordinating this project. Two students completed undergraduate thesis. Four students completed summer undergraduate research experience terms. The project provided employment for two research assistants, two lab technicians, and a project co-ordinator. There has been outreach via conferences in the areas of One Health, agriculture and veterinary science and further outreach is planned among the scientific community. All study participants were provided with a report of their water sampling results. These reports were also distributed to the relevant health inspector and veterinary clinic if applicable. Recommendations based on the study findings will be shared with stakeholders at two meetings in the fall, and via reports to policy stakeholders.

Methodology

Please provide a narrative describing the methodology and facilities that were used to execute and complete the project. Use subheadings as appropriate.

RESPOND BELOW

This research project involved the collaboration of an interdisciplinary team of researchers based at the University of Calgary and the University of Alberta, University of Victoria and the Alberta Public Laboratories (APL) (previously Alberta Provincial Laboratory for Public Health). Individuals responsible for specific project objectives were identified at the outset of the project.

Processing of water samples at APL Calgary:

Groundwater samples submitted to the APL in Calgary Alberta are analysed using routine processes for detecting the presence of microbial contamination (total coliforms and *E. coli*). APL is an ISO 17025 accredited government funded laboratory providing centralized water quality testing services to residents of Alberta. APL Calgary processes samples from the southern third of Alberta, the area south of Red Deer.

Private sample collection (all retrospective and prospective sampling): Water sampling kits are provided for well owners by the local health authority. A sampling kit contains a sterilized bottle (250 mL) containing sodium thiosulfate, a requisition for collecting personal/well information (including GIS

coordinates), and sampling instructions. The procedure for collecting a water sample is outlined (Appendix 1).

Recruitment study sample collection (objective 2): The research team collected water samples as part of the recruitment study and the virology study. Researchers responsible for collecting the water samples followed the same guidelines provided in the water sampling kits provided to well owners (Appendix 1). Samples were stored in a cooler and delivered to APL within 24 h of collection of the first stored sample. The one difference in the sampling procedure in the recruitment study compared to a routine submission as outlined above was that two 250 mL bottles of water were collected instead of one. This was to allow for the processing of the extended pathogen screening tests on each water sample.

All sample analysis: To determine the microbiological quality of the water sample as per Canadian drinking water guidelines, 100 mL of the submitted water sample was tested for total coliforms and *E. coli* using presence/absence defined substrate methodologies (i.e., Colilert®, IDEXX Laboratories Inc.). The remainder of the water sample was stored at 4°C. Colilert® samples were incubated at 35°C ± 0.5°C for 24 hours and assessed for total coliform contamination (yellow color change) and *E. coli* contamination (fluorescent)

*Extended testing for Enterococcus via Enterolert***:** Samples collected by the research team for the recruitment study and virology study were also tested for the presence of culturable *Enterococcus* spp using the Enterolert kit (Enterolert®, IDEXX Laboratories, Inc).

Extended pathogen screening: Water samples were immediately processed for molecular testing by filtering 100 mL of the water through 0.22um polycarbonate filters. Filters were stored and frozen at -86^{o}C until processed for DNA extraction. Filters were transported to APL in Edmonton for further analysis by the research team at the University of Alberta. The methodology for DNA extraction is outlined below.

STEC Study: Detection, serotyping and mapping of STEC:

The methodology as described is reported in draft manuscript(s) that will be submitted for publication in peer reviewed journals (See draft publications under section E – project outputs)

Objective 1, Task 1.1 and 1.2, Objective 2, Task 2.1 and Objective 3, Task 3.1 and 3.2

Detection of STEC: For the detection of STEC, *E. coli* positive samples collected from the routine well water samples submitted to APL from 2004 – 2016 were utilized. From March 2004 to May 2015 *E. coli* positive well water samples were cryopreserved and archived. These samples are referred to as retrospective samples and were used to address the objectives set in Task 1.1. Task 2.1 utilized water samples submitted routinely to APL between 2015-2017. These samples were not subjected to freezing or storage conditions before analysis and are referred to as prospective samples. The methodology used for identification of STEC was the same for both retrospective and prospective samples. Samples were analysed by quantitative polymerase chain reaction (qPCR) against the *stx1* and *stx2* genes as a proxy screen to determine the potential presence or absence of STEC.

The *stx* positive samples identified via qPCR were plated onto CHROMagar™ STEC agar (CA-STEC) to attempt to isolate individual STEC strains. Presumptive STEC isolates were recovered and tested for the presence of *stx1* and *stx2* by colony qPCR to determine presence/absence of STEC-related toxin genes in each isolate. If multiple *E. coli* isolates from one sample were *stx* positive via qPCR then *stx* positivity from each respective isolate was aggregated and attributed to its corresponding sample.

Serotyping of STEC: This was followed by (GTG)5 rep-PCR analysis on STEC isolates to: i) characterize genetic diversity of isolated strains within each of the water samples; and ii) identify the repertoire of unique STEC strains observed in private well water samples so as to minimize clonal

representation of isolates sent to the Canadian National Microbiology Laboratory for serotyping. Isolates were subsequently 'fingerprinted' by high-resolution capillary electrophoresis DNA-fragment analysis using the QIAxcel® Advanced system. Unique genetic isolates then tested via a Vitek® Automated Bacterial Identification System (bioMerieux, Marcy-l'Étoile, France) to confirm that the bacterial isolates were truly *E. coli* and confirmed isolates were then sent for serotyping to the Canadian National Microbiology Laboratory.

Shiga toxin gene (*stx*) positivity rates per 10 000 submitted non-municipal drinking water samples were calculated as a proxy for STEC occurrence rates within non-municipal drinking water sources in southern Alberta. Negative binomial regression was used to test the relationship between time and annual *stx* positivity rates in submitted samples. The year 2010 was used as a referent as this is the year with the lowest number of positive samples. Years without complete submission data (2004 and 2016) were not considered as referents. A monthly breakdown of STEC positivity was also analyzed from the aggregate dataset.

Spatial and temporal analysis of STEC: In order to examine the spatial prevalence of STEC in groundwater STEC occurrence was mapped based on geolocation data provided by well owners and collected on the requisitions used by APL. The geographic locations of submitted *E. coli* positive drinking water samples with complete Alberta Township System (ATS) information, along with their respective *stx* positivity as determined by *stx1*/*stx2* qPCR analysis, were represented. The geographic location of each of the samples for which viable STEC was recovered and along with the corresponding serotype of each STEC was mapped. To investigate spatial clustering of STEC occurrence and identify any geographic regions of potentially increased risk of *stx* contamination of groundwater wells in southern Alberta, *stx* occurrence was analyzed via a Bernoulli spatial scan statistic or the space-time permutation model Kulldorff scan statistic as appropriate.

AMR study: Detection of AMR, identification of ESBLs, and mapping of AMR

Objective 1, Task 1.3, Objective 2, Task 2.4 and Objective 3, Task 3.1 and 3.2

The samples utilised for the detection of AMR were the same archived samples and prospective samples as described above. Samples submitted to APL between August 2006 and August 2016 were utilised. There were 67,339 samples submitted during this time period, of which 1328 samples were positive for *E. coli* (1.97%). These samples addressed task 1.3. An additional 121 *E. coli* positive samples were collected prospectively, of which three were rejected because the date of collection was not interpretable. These samples addressed task 2.4.

One mL of each *E. coli* positive sample was enriched in 9 mL of tryptic soy broth (TSB) for 16-18 hours. Samples were vortexed and approximately 50 µL was streaked to isolation on an X-Gluc agar plate (Dalynn Biologicals, Calgary, AB, Canada) before incubation at 35±2°C for 18-24 hours. A blue precipitate on the X-Gluc agar plate indicated the presence of *E. coli*, and up to 20 presumptive *E. coli* colonies were picked from each sample. Isolates were stored in 96-well plates and screened for AMR using an agar screen plate method including one plain MacConkey plat and seven supplemented with antimicrobials (gentamicin 8mg/mL, streptomycin 32mg/mL, ampicillin 8mg/mL, nalidixic acid 4mg/mL, sulfamethoxazole 128mg/mL, cefoxitin 32 mg/mL, tetracycline 4mg/mL). Each agar screen plate was inoculated with 40 presumptive *E. coli* isolates and 8 control strains using a 48-pring replicator. Agar plates were incubated at 35±2°C for 18-24 hours and isolates with growth on one or more antimicrobial supplemented agar plates were streaked to isolation on MacConkey agar, then Sheep's Blood agar plates. Where multiple resistance profiles were observed on antimicrobial-supplemented media, one isolate of each resistance profile was selected for further workup. To confirm the species of each presumptive resistant isolate, API®20E biochemical test strips (Biomerieux, St Laurent, QC, Canada) were inoculated

according to the package insert. Results were interpreted using the apiweb™ identification software with an inclusion criteria of ≥90% *E. coli*.

Broth microdilution antimicrobial susceptibility testing was performed on *E. coli* isolates to determine the minimum inhibitory concentrations (MICs) to 14 antimicrobials. The Gram-negative National Antimicrobial Resistance Monitoring Systems (NARMS) Sensititre™ test panel CMV3AGNF (Thermo Fisher Scientific, Burlington, ON, Canada) was inoculated with a single *E. coli* isolates as per the package insert and results were interpreted based on the Clinical Laboratory Standards Institute (CLSI) guidelines by the Sensititre™ SWIN® software system (Thermo Fisher Scientific, Burlington, ON, Canada). In cases where the software could not interpret the MIC values (azithromycin and sulfisoxazole), associates at the Public Health Agency of Canada were consulted for expert opinion on how Canadian surveillance systems interpret MIC values. When multiple *E. coli* isolates were detected from an individual water sample, each was considered distinct if resistance to a single antimicrobial differed by two or more dilutions.

Quality control: Each set of agar screen plates had negative controls including TSB alone, to ensure no bacterial contamination, and ATCC 25955 *E. coli*, a standard strain with known MIC values. Positive controls included two non *E. coli* and four *E. coli* strains with known MIC values for each of the antimicrobials on the agar screen and NARMS Sensititre™ panel. Antimicrobial resistant laboratory strains *Pseudomonas aeruginosa* URH5057 and *Klebsiella pneumonia* IS-625 were also used as positive controls. One isolate form every 20 samples with no growth on the agar screen was tested for resistance to ensure accuracy of the screen process. Laboratory strain ATCC 25955 *E. coli* was used as a quality control strain with each batch of NARMS Sensititre™ panels and API®20E biochemical test strips.

ESBL and Phylogenetic Group Testing: Isolates flagged as potential ESBL producers that conferred resistance (or were intermediate) to a third-generation cephalosporin, ceftriaxone, via the SWIN® software system (Thermo Fisher Scientific, Burlington, ON, Canada) were flagged. AmpC- and ESBL-producing isolates were distinguished using Mast® 68C ESBL and AmpC detection kit (Alere, Ottawa, ON, Canada) containing four disks containing cefpodoxime (10 µg) in combination with clavulanate (ESBL inhibitor) and/or cloxacillin (AmpC inhibitor) Mueller Hinton agar was inoculated according to the manufacturer's recommendations and the CLSI guidelines. Phylogenetic groups of the 27 potential ESBL-producing *E. coli* isolates were determined as per the triplex PCR scheme described by Clermont *et al.* (2000).

Spatial and temporal analysis of AMR data: E. coli positive samples screened as above for AMR that had Alberta Township System (ATS) locations were used for spatial clustering and temporal analysis (n = 741/1241). The ATS system consists of quarter, section, township, range and closest meridian on its eastern side. The resolution of the ATS system is approximately one quarter section or 800x800 meters (Government of Alberta 2020; Invik et al., 2017). Data values from the ATS system were converted to latitude and longitude coordinates for the centroid of the quarter section using an Excel template created for previous work in our lab (Invik et al., 2017). Data was visualized with the North American Datum of 1983 coordinate system and projected to the North American Datum 1983 10 TM AEP Forest coordinate system.

All maps were created in ArcGIS (version 10.4.1, Esri Inc. 2015). Chloropleth maps were created with sample level data aggregated to a map of the Canadian Census Region from 2006. The maps show the number of AMR positive wells as a proportion of the total number of wells positive for *E. coli*. Maps were created for the proportion of wells positive for each of the following variables: AMR, MCR (multi-class resistance), and resistance to aminoglycosides, cephalosporins, chloramphenicol, macrolides,

penicillins, (fluoro-)quinolones, sulfonamides and tetracyclines. Positive well maps were created using binary point data displayed as individual well samples positive or negative for AMR *E. coli*.

To determine whether spatial clusters existed, we used a purely spatial analysis with a Bernoulli probability model as our input data was binary point data (Talbot et al., 2015) in SatScan™ (version 9.4.4, SatScan, 2016). Cluster results were exported as shapefiles from SaTScan™ and added to existing chloropleth maps or point data maps where appropriate in ArcGIS. Clusters with a p-value < 0.05 were considered to be statistically significant.

Edward's test of seasonality was used to determine if there was a significant seasonal effect in the data (WINPEPI version 11.65). Seasonal trend loess decomposition was used to separate seasonality from overall long-term trend, and noise, or random fluctuations using R (version 2.14.0, R Development Core Team 2011). Seasonality has previously been detected in *E. coli* positivity in rural well water in Alberta (Invik et al., 2017). Using a numerator that is seasonal when trying to detect seasonality will effectively cancel out the effect. For this reason, the numerator used was all private well water tests submitted to APL Calgary for the same time period for both Edward's test and seasonal trend loess decomposition.

Recruitment study: Development of a questionnaire (farm survey)

Objective 2, task 2.2

The methodology used to recruit participants and collect questionnaire data and water samples is described in Caffrey et al. (2020) and is reproduced here for convenience.

Questionnaires tailored to target three different livestock production systems in rural Alberta were designed as a collaboration between the Public Health Agency of Canada, Alberta Health Services, Alberta Agriculture and Forestry, the University of Alberta, and the University of Calgary. In conjunction with CIPARS/FNC, three veterinary practices in central Alberta were recruited to assist with targeting 30 producers in each of three livestock production systems (broilers farms, feedlots, cow/calf operations). Veterinarians administered the questionnaire and collected well water samples during routine visits from clients willing to take part in the survey. Veterinarians received monetary compensation to recruit their clients, fill out questionnaires and to collect and submit water samples. Their clients received a rebate of $100 on their veterinary expenses for completing the questionnaire and submitting a well water sample. A consent form outlining the purpose of the survey and providing the contact information for the research team was provided to each client. Recruitment by veterinary practices took place in 2015-2016. Due to lower than anticipated levels of recruitment through veterinary practices, other types of recruitment were added that were different from the usual CIPARS/FNC recruitment process. A second questionnaire was developed to administer to acreage owners living in rural areas and using well water as their primary source of drinking water. A number of livestock owners and acreage owners were recruited by advertising through the Association of Alberta Agricultural Fieldmen's newsletter and through the Working Well initiative run by the Alberta government. Social media and an online classifieds website were also utilized to advertise the survey. Acreage owners or livestock owners not recruited through the veterinary practices received a $100 gift card for completing the questionnaire and submitting a water sample. Recruitment was completed in March 2017. Where recruitment was not through a veterinary practice, questionnaires were administered over the telephone, via e-mail using fillable PDFs, and in person. One trained person administered the questionnaire (NC). Questionnaire content was designed to collect pertinent information from producers regarding farm size and makeup. Information was gathered regarding water sources used, presence of animals in the vicinity of the water source and management of the water source. Questions regarding the operational management of the farm included factors such as manure management, pest control, use of disinfectants and use of antimicrobial products, as well as information regarding the drinking water preferences of the owner. For the questionnaire administered to acreage

owners, questions relating to farm operation and management were removed. However, information on proximity to potential risk factors such as the presence of livestock and manure remained in the questionnaire.

Questionnaire results were analysed in conjunction with information available for the water well drilling reports and the results of the water sample test taken on the day the questionnaire was administered. The well ID was available for 70 wells from the recruitment study. The well ID allowed access to the well drilling report from the Alberta government's "Alberta Water Wells" database. The drilling reports include a number of variables that have the potential to impact well contamination rates. Variables extracted included type of work (ie new well or old-well test, or deepened well), year drilled, total depth drilled (meters), static water level (meters), water removal rate (L/min), draw down (meters), drilling method, surface casing type, well casing type, annular seal type, and elevation of the well head (meters). Drawdown and water removal rate were only available on 24 of the available drilling reports.

Well reports also usually include a formation log which gives the depth from ground level coupled with a lithology description for each layer that the driller encounters during the drilling process. This information was used to calculate hydraulic resistance for the wells. Hydraulic resistance is a measure of the resistance of soil environment to vertical flow of water and has been previously used to assess groundwater vulnerability. A vulnerability index was created by compiling a list of hydraulic conductivity values for the various materials encountered by drillers in Alberta, using the midpoint of existing hydraulic conductivity ranges. Using these values a hydraulic resistance was calculated for wells where appropriate data was available. Transmissivity, a measure of the overall capacity of an aquifer to produce water was also calculated for each well. This information was used in conjunction with data collected via the questionnaire to characterise the wells participating in the recruitment study for which water well drilling logs were available.

The association between the presence of total coliform positive test results and potential risk factors identified in the questionnaire were examined using Pearson Chi^2, Fisher's Exact test or as unconditional associations in logistic regression models. Associations between premises type and factors such as the frequency of testing, purchasing of bottled water, and concern about contamination were examined using logistic regression. Responses to a question asking if respondents were concerned about their water well becoming contaminated were also treated as an outcome of interest in unconditional logistic regression models.

Virology study:

Sampling methods and collection: The innovative water sampling device (WSD) was developed (Figure 1). The device was distributed to the participants who were trained to operate the WSD to take a large volume of groundwater samples (500 litres) monthly from their wells for consecutive 12 months. Five-hundred litres of water was passed through electropositive filters (NanoCeram VS2.5-5 (Argonide Corp, Sanford, FL, USA)). The filters were collected on a pre-arranged schedule and shipped in cool condition from all sites across the province to the testing lab within 24 hrs. An additional 200 ml of matched tap water samples were collected and shipped simultaneously to the lab for bacterial testing.

The sampling schedule for each participant site was once per month for consecutive 12 months. A project coordinator visited each site at the sample collection day to ensure the standard sampling procedure was followed and to answer any questions in assistance with sample procedure. A great effort was made by the team to complete sample collections of high quality from 50 participants across the province for 2.5 years.

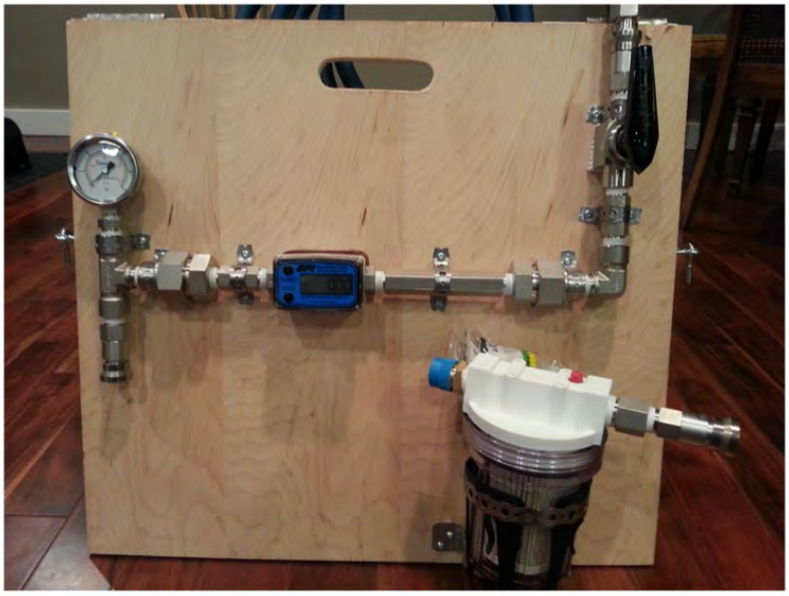

Figure 1: Water sampling device (WSD) created for the project and used for collecting a large volume of well water for enteric virus testing.

Laboratory testing: A pre-analytical process for eluting and concentrating the viruses from the filters was developed (Appendix 4). This procedure was labor intensive. Viruses retained by the filter were eluted using a large volume of beef buffer, flocculated and centrifuged to get a pellet of concentrated viral sample.

- Viral nucleic acid (NA) extraction was performed from concentrated samples. The NA extracts were aliquoted and stored in -70 C freezers until testing.
- One aliquot of concentrated sample was used in cell culture for cultivable viruses, i.e. rotavirus, adenoviruses, enterovirus and reovirus. The cell lines of BMG, and MA104 were used for these viruses in culture. Cell culture was carried out for 2 passages for each sample in consideration of limited viral particle if present in the samples.
- Real-time quantitative PCR (for DNA viruses) and RT-PCR (for RNA viruses) assays were conducted to detect viral nuclei acids in the samples. This quantitative method has high analytic sensitivity and fidelity. Panel testing included 7 enteric viruses, most of them human viruses. The standard curve was prepared and integrated to the assays for quantifying the level of viruses in groundwater samples. The copy number per litre of water was expressed as readable outcomes, which reflects the levels of specific viruses in the samples.
- Integrated cell culture with qPCR (ICC-qPCR) was initially developed by Dr. Pang's lab and has been used and cited in numerous studies. This innovative method increases the sensitivity of classical cell culture and the end point reading of cytopathogenic effect (CPE) of viruses growing in the cells significantly. ICC-qPCR allows detection of infective viruses with known levels in a post-culture assay.

- Examination of TC, *E. coli* and *enterococcus* were performed as described above.

Extended pathogen screening and contamination persistence study
Objective 2, task 2.5

Task 2.5. Identification of repetitive well failures and perform extended pathogen testing (*Salmonella, Campylobacter, Cryptosporidium, Giardia*).

The methodology as described is reported in draft manuscript(s) that will be submitted for publication in peer reviewed journals (See draft publications under section E – project outputs)

Molecular testing methodology: Extended pathogen testing in the form of a molecular prescreen was undertaken on every sample submitted as part of the recruitment study, and on *E. coli* positive samples used in objectives 1 and 2 submitted as part of the prospective study. Cryptosporidium and Giardia were not included in this screening for reasons outlined above. The pathogens for which all samples were tested included *E. coli*, *Enterococcus* spp., *Salmonella* spp., *Campylobacter* spp., *Arcobacter butzleri* and general Bacteroides.

Bacteria DNA was extracted from the stored filters using the EPA Method 1611 DNA extraction method. Samples were analyzed for a variety of microbial targets using quantitative polymerase chain reaction [qPCR] (Table 1). All qPCR assays were run on an ABI 7500 Fast cycler under the following cycling conditions: 95°C for 2 minutes followed by 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. All reactions were performed using either ABI Fast Advanced Master Mix (Thermo Fisher) or PrimeTime Master Mix (Integrated DNA Technologies) and supplemented with BSA (200 □g/mL). Five microliters of each DNA extract was used as a template in each qPCR reaction. All the direct quantification methods were run against standard curves (50,000 – 0.5 copies/reaction) generated by serial dilution of positive control plasmids. The *Enterococcus* molecular assay (Entero1) was performed as described in EPA Method 1611 (USEPA 2012). A PCR inhibition control as part of EPA Method 1611 was used to determine if negative results were due to PCR inhibition. This internal control assay targets the salmon sperm DNA (Sketa) used in the DNA extraction buffer. Any PCR reactions in which the Sketa C_T value was shifted by >3 C_T relative to control reactions were deemed to be inhibited. Primers and probes for each assay are described in the references in Table 1, except for *uidA*, which utilized the probe from Taskin et al. (2011) and the in-house designed primers *uidA*-F – 5'-cgcaaggtgcacgggaata-3' and *uidA*-R – 5'-caggcacagcacatcaaagaga-3'. Where appropriate, limits of detection (LODS) were determined for each target are provided (Table 1).

Table 1: PCR markers used in this study

Source	Gene Target or Marker	LOD_{95} (upper limit/lower limit) with copies/reaction	Primer/Probe concentration (nM)	Reference
Escherichia coli	*uidA*	5.90 (4.33/8.03)	450/125	This study and Taskin et al., 2011
Salmonella spp.	*invA*	4.94 (3.23/7.56)	450/125	Daum et al., 2002
Arcobacter butzleri	*hsp60*	8.22 (6.09/11.10)	300/100	de Boer et al., 2013
Campylobacter spp.	*16SrRNA*	11.96 (6.89/20.73)	300/100	Van Dyke et al., 2010

General *Bacteroides*	GenBac3	6.91 (4.03/11.83)	400/125	Siefring et al., 2008
Enterococcus spp.	Entero1	N/A[a]	1000/80	USEPA, 2012
Oncorhynchus keta	Sketa	N/A[a]	1000/80	USEPA, 2012
[a] The Entero1/Sketa assays use a relative quantification method and as such does not have an LOD_{95} value like the other direct quantification methods. (LOD - Limit of Detection)				

qPCR test results from each study were matched with the APL sample ID and entered into Excel. qPCR test results were merged with bacterial test results and information regarding the well taken from the APL requisition form submitted with the water sample.

The results of the qPCR testing were examined in conjunction with the results of the standard tests for each sample collected as part of the recruitment study, and a subset of the *E.coli* positive sample submitted to the APL over the corresponding study period. Test results were evaluated descriptively at the sample level and at the well level in the case of the samples from the recruitment study. Samples were analyzed to assess the proportion of samples positive for each molecular indicator in the prospective study versus the recruitment study. Associations were assessed using two sample tests of proportions, Pearson's chi^2 or Fisher's Exact test, logistic regression and using Cohen's Kappa statistic.

The persistence of contamination in well water was assessed by examining decomposition and transitional probabilities for wells that were positive for *E. coli* via culture and wells that were positive for *Enterococcus*, *E. coli* and *Bacteroides* via qPCR. All statistical analyses were undertaken in Stata 15. GIS was used to map the location of six persistently contaminated wells in relation to factors of interest such as the underlying bedrock formation, soil type and drainage properties, whether the well was located in a no till farming or irrigation area, and geological suitability for waste. Two draft publications are in the final stages of preparation.

Objective 3

This objective was addressed separately for the STEC and AMR portions of the study as described above. GIS information was also used to map wells that were examined with regards to the presence of persistent water well contamination as detected via the molecular pre-screen (task 2.5) and for two sub-projects addressed under objective 6 (vulnerability mapping study, rare event detection study).

Objective 4

Source tracking study

The methodology used for qPCR for Bacteroides is outlined under Objective 2 Task 2.5. Where the general *Bacteroides* marker was positive in a sample, the sample was further tested for the presence of markers indicating whether the faecal contamination was from humans or cattle. This included the HumM2 marker and HF183 marker for human *bacteroides* and the cattle specific marker CowM3. The HumM2 marker (Shanks et al., 2009) is specific to bacteria found in human feces and its presence is indicative of human sewage contamination. The primer/probe sequences are as follows: HumM2-F (5'-CGTCAGGTTTGTTTCGGTATTG-3'), HumM2-R (5'- TCATCACGTAACTTATTTATATGCATTAGC-3'), HumM2-P (5'-FAM- TATCGAAAATCTCACGGATTAACTCTTGTGTACGC-TAMRA-3'). The CowM3 marker (Shanks et al., 2008) has been modified from the original published marker. We designed a new probe (CowM3-P2) within the same amplicon, but maintained the original primers as described by Shanks. Presence of this marker is indicative of bovine fecal contamination. The primer/probe sequences are as follows: CowM3-

F (5'- CCTCTAATGGAAAATGGATGGTATCT-3'), CowM3-R (5'- CCATACTTCGCCT GCTAATACCTT-3'), CowM3-P2 (5'-FAM- GGAAAGCAGGAACTTA-NFQMGB-3').

Objective 5

Task 5.1. Enroll 500 livestock producers in this study.

The livestock producers sampling frame used for this project was based on the Alberta Well Water Information Database and the Baseline Well Water Testing Program. Wells were classified on the basis of: watershed in which they are located; declared use for household or livestock purposes; and date of information (last 10 years). From this subset, 2000 participants were randomly selected to receive mail out questionnaire packages that included a cover letter explaining the research and soliciting their involvement, a paper questionnaire, a card indicating a website address and QR code for digital access to the questionnaire, and a postage-paid return envelope for the paper questionnaire. In addition, a request with instructions to submit a drinking water well sample to the Provincial Laboratory of Public Health and Alberta Centre for Toxicology (ProvLab) was included. These households also received two reminder cards at four week intervals.

Task 5.2. Develop and implement questionnaires, focus group discussions, and facility mapping that will be used to investigate perceptions (via probit analysis) and develop qualitative objective assessments relating to water and public health perception, water and manure management biosecurity procedures, and zoonotic disease mitigation strategies. These methodologies and results are reported in Munene et al, (2019) and Munene et al (2020).

The questionnaires were developed following a standard approach:

a) Guided by the hypothesis, develop the conceptual, theoretical, and statistical models
b) Decide what variables are needed to test sufficiently the hypothesis based on the theoretical model, including ownership of livestock on site
c) Decide on the nature of the data to collect to populate each variable (continuous, discrete, etc.)
d) Develop questions that will collect the data identified in the previous step
e) Cluster questions into appropriate categories
f) Trial the questionnaire where possible (many of the questions have been asked in slightly different contexts in previous or other ongoing studies)

In addition to the mail outs, the following Watershed Planning and Advisory Councils (WPAC) and one forage association were contacted; all agreed to pass on a request for study participation to their members: Athabasca; Battle River; Beaver River; Bow River Basin; Mighty Peace Watershed Alliance; Milk River; North Saskatchewan Watershed Alliance; Oldman; Peace Country Beef & Forage Association (PCBFA) Whole Farm Water Planning Day; Red Deer River; South East Alberta Watershed Alliance.

The following county councils were also contacted with a request to post our request for study participation on their website. The first five agreed; the others either did not respond or declined: Clear Hills County; County of Forty Mile No. 8; Lacombe County; MD of Bighorn No. 8; MD of Fairview No. 136. The following either did not respond or declined: Athabasca County; Beaver County; Big Lakes County; Brazeau County; Camrose County; Cardston County; Clearwater County; County of Barrhead No. 11;

County of Grande Prairie No. 1; Cypress County; Flagstaff County; Lac La Biche County; Lac Ste. Anne County; Lamont County; Leduc County; Lethbridge County; MD of Acadia No. 34; MD of Foothills No. 31; MD of Lesser Slave River No. 124.

Participants who responded to the initial questionnaire mail outs (either through recruitment from the Alberta Water Well Identification Database (AWWID), WPACs, Working Well Program Workshops or responded to invitations online or at local stores) were contacted and asked to participate in a semi-structured focus group discussion c. August, 2017. Participants of the focus group discussions were also requested to submit a well water sample for testing. They also were asked to complete a second shorter questionnaire addressing perceptions of water quality.

Focus group discussions: The core qualitative model with which to process and analyze information from focus groups was identified as thematic analysis. For details of this approach, see Guest et al. (2012). Transcription and analysis was conducted by the PhD student associated with the project. Data were anonymized and all participants provided their informed consent to have their conversations recorded and their data analyzed for this study.

Facility mapping: Facility mapping was achieved by asking respondents to indicate on the questionnaire the presence of various structures (e.g., barn, pen, house, well, manure pile) as well fill in a matrix of distances between those objects. These coordinates were to be used to construct geospatial variables for analysis.

Perceptions, Objective Assessments: Perceptions and qualitative objective assessments (e.g., choices and preferences) were developed primarily through use of standard regression analysis techniques including probit and analysis, supported by summary statistics and tests of significance. Recommendations regarding biosecurity management procedures and zoonotic disease mitigation strategies reflect results from those analyses.

NOTE: water collection and testing was as per other collection for this study described above (page 12).

Objective 6: Use information gained from the study to inform decision makers on the implications for human, animal and environmental health (e.g. water testing policies (microorganisms to test, lack of regulation of testing for private drinking water), risk maps, livestock biosecurity and other mitigation strategies). (Everyone involved in interpretation).

A number of sub-projects have been completed from which information will be used to inform decision makers on the implications for human, animal and environmental health. These sub-projects have sought to create risk maps (vulnerability mapping study, rare event data study), engage with stakeholders by creating data visualisations programs (Tableau interface), and assist environmental health inspectors in understanding risk factors for water well vulnerability (VRAT and faucet study). The methodologies for these projects are outlined below.

Vulnerability mapping study: The findings of this study have been published (VanStaden et al., 2019). The methodology reported in the publication is outlined here. An assessment and mapping of groundwater vulnerability to bacteria in Alberta was undertaken. GIS tools were used to identify and model key vulnerability factors specific to microbial sources and subsurface transport mechanisms. Intrinsic vulnerability maps for *E. coli* were generated for shallow aquifers in Alberta for 2012. The study area

coincided with the agricultural regions of the province where appropriate soil property information is available. Six vulnerability factors (soil moisture, pH, soil texture, soil organic matter, hydraulic resistance and precipitation) were selected. The seasons were defined as the cold season (October – March) and the growing season (April – September). A GIS layer for each factor was created, with each layer adjusted to reflect the relative impact of that factor on bacteria survival using vulnerability indexes. The vulnerability index reflects the relative influence a factor's variability has on the overall aquifer vulnerability based on literature, data distributions and existing regulations. GIS layers representing high and low vulnerability were created, and vulnerability indexes were normalised to give each factor equal weight. Layers were then summed in GIS to produce an intrinsic bacterial vulnerability map. The list of ranges and corresponding vulnerability indexes for each factor are available (VanStaden et al, 2019).

Water sample test results (n = 8,610) from the APL for the sampling period identified were used to assess correlations with the presence of *E. coli* (n = 155) in the wells. Each layer was compared to *E. coli* detections with weights assigned based on the significance of the correlation. Kruskal-Wallis tests were used to compare the vulnerability values and to compare the overall vulnerability maps.

VRAT Study:

The methodology as described is reported in draft manuscript that will be submitted for publication in peer reviewed journals (See draft publications under section E – project outputs)

The vulnerability risk assessment tool (VRAT) for wells assesses four contamination pathways using a series of questions to identify if there is the potential for intrusion to occur. The four sections relate to well head construction, local aquifer susceptibility, and nearby point source contaminants. Wellhead assessment identified whether features such as surface grading, potential for flooding overtop of the well head and well cap integrity could compromise the well. The well construction is assessed to identify potential for intrusion through the annular space of the well. The aquifer assessment involves analysis of the potential for intrusion through the underlying geology by analysing the hydraulic conductivity of the lithology from the well log to the depth of the well screen. A residence time less than 90 days is used as an initial vulnerability benchmark. The most conservative hydraulic conductivity values from the following sources were used (Clapp and Hornberger, 1978; Freeze and Cherry, 1979; Heath, 1983; Brassington, 1990; Domenico et al., 1998). The distance to the well from key points of potential faecal contamination such as livestock yards, septic tanks, surface water and manure storage was evaluated to determine whether these facilities met the setback distance outlined by The Environmental Public Health Field Manual for Private, Public and Communal Drinking Water Systems in Alberta (Government of Alberta 2004). Responses to the screening questions were assigned a level of threat with the possible outcome being: no threat, potential threat, and/or direct threat. Threat levels correspond to the likelihood of contamination and provide scalable actions for owners to implement to further assess or reduce risk and explain intrusions pathways.

The VRAT was applied to wells on 40 sites in southern Alberta between May 2016 and December 2018. These sites included farms and acreages. Well owners were recruited based on their participation in the larger recruitment study regarding well water, and by word of mouth recruitment. Participation in this study was voluntary. The four sections of the VRAT questionnaire were completed for each site. Most of the sites had one well. However, there were two sites that had multiple wells all feeding into one source. For each well at these sites, a separate VRAT questionnaire was completed. Then, to combine these assessments into one, the highest threat of each question was chosen. For example, if one site had

four wells, and the question about the tightness of the well cap indicated a no threat for two wells, a potential threat for one well, and a direct threat for one well, the overall assessment for all four wells indicated a direct threat for this question. The VRAT was considered in conjunction with the results of the water testing carried out simultaneously using standardized collection and testing methods as outlined previously.

A single investigator conducted the VRAT assessment for each of the 40 sites. Well drilling logs were obtained from the Alberta Water Wells Database (Government of Alberta, 2019b). Well information that was used to help complete the assessment was gathered from the well log. This included information related to the well screen depth, lithology and construction methods. The investigator visited each site to review the wellhead and point source assessment sections with each well owner. Questions were answered using the appropriate outcome designation based on the findings (direct, potential or no threat).

Data were analyzed using Stata 15 for Windows (StataCorp. 2017). Statistical significance was set at $p<0.05$. Agreements between VRAT and bacteriological results were categorized into contingency tables. These agreements were analyzed using Cohen's Kappa statistic and Kendall's Tau rank correlation coefficient. Sensitivity, specificity, false positive rates, and false negative rates were also calculated. For this analysis, two levels of threat were used: no threat and threat. Logistic regression was used to test for various associations among the 40 wells included in this study and their microbiological results. The parameters examined included age of well, depth of the well, depth of screen, and residence time. The distances to septic tank, septic field, livestock, manure storage, and surface water were also examined.

Faucet Study This study was completed on 21 sites from which positive water samples were taken over the duration of the study. The purpose of the study was to assess whether the point of sample collection, i.e. the faucet, used for the original samples may in some way have contributed to the positive test results at that site. An intervention study was designed to test this hypothesis. The intervention involved choosing an 'optimal' faucet at each site, and/or the use of a disinfection procedure on the faucet prior to taking the water sample. The methodology for this study is outlined (Appendix 3).

A questionnaire was administered in conjunction with the intervention to collect pertinent information such as the location of the faucets, the presence of an aerator, flex hose, filters, water treatment, and cisterns. It also collected information as to whether there was any staining, film or debris on the faucet, aerator or sink fixtures. The questionnaire is provided (Appendix 3). An inspection camera designed for use examining enclosed pipe systems was used to inspect faucets and take photographs.

Samples were considered as positive for contamination if the sample was positive via bacteria culture (total coliforms or *E. coli*) or via qPCR (*E. coli, Enteroroccus, Bacteroides*). Two sample tests of proportions, Wilcoxon signed rank test and chi square statistics were used to evaluate whether there was a difference in the level of contamination before and after the disinfection procedure.

Creation of a Tableau interface: Tableau is an exciting visualization tool that allows a user to produce an interactive dashboard of one or more datasets. These dashboards can be distributed publically or security settings can be used to limit the audience to a small group. A dashboard was produced in Tableau to allow the research group to explore two related sets of data, well water bacterial test results, combined with the results of the questionnaire administered during the recruitment study. These separate spreadsheets

were cleaned and reorganized for use in Tableau. The two spreadsheets were imported into Tableau and a union between the two sheets was created, using the premises ID as the key. In development mode Tableau consists of a number of tabs on the bottom of the program, similar to an Excel workbook. In the case of Tableau, these tabs can represent worksheets, dashboards or stories. The worksheets are single elements, a table or a map or a chart. The elements created in the worksheets are then combined into a dashboard. Each dashboard can contain a number of elements including charts, maps, statistics and listing of raw data. One or more elements on each of these dashboards can be used to filter the information displayed by other elements. For instance on a number of dashboards, a map is used to display each of the premises. Clicking on a premises on the map allows the rest of the information displayed on the dashboard to be filtered to display only the information on that single premises.

One of the great features about Tableau is that dashboards can be a published individually, but they can also be combined together in a Story, allowing a progression of information to be provided in a meaningful order. In the process of creating a story, individual graphs, charts and maps are created on worksheets. On the left-hand side of the developer's screen are a list of dimensions and measures that are either included in the original excel spreadsheet, or are a calculation created within Tableau. Dimensions are fields such as premises type that cannot be aggregated or used for mathematical calculations. Measures are elements such as the number of broiler chickens on a premises that can be aggregated and used for mathematical calculations. Dimensions and/or measures are pulled into the middle and an appropriate graph/table/map is chosen to display these elements. Individual worksheets are then pulled onto a dashboard and resized and arranged to create an array that is functional and useful. Certain elements in the dashboard can have filters attached to them and are then used to filter other elements in the display. A series of dashboards can be combined into a story, with leading and following pages describing various elements of the story.

Statistical analysis and modelling of rare event data: The goal of this study was to find factors which could cause bacterial outbreaks in the groundwater of Alberta by taking samples from wells and evaluating both the characteristics and locations of those wells and environmental factors leading up to the bacterial bloom. The report submitted in partial fulfillment of the academic requirements of the co-op term for the BSc student undertaking this analyses is available (Appendix 6). This study utilised the samples collected as part of the recruitment study, resulting in analysis of 705 samples taken from 98 premises. Environmental data for 48 weather stations near the sample points was collected from Environment Canada (Environment Canada, 2019) and imported to Microsoft Excel for the years 2015 -2017. Temperature and precipitation data for the seven days proceeding the sampling date was used to determine the environmental factors that may be correlated to bacterial contamination. The maximum temperature (Max), minimum temperature (Min), 7, 3, 2, and 1 day mean average temperatures (T7, T3, T2, and T respectively) were collected for each sample point. Additionally, the 7, 3, 2, and 1 day mean average precipitations (P7, P3, P2, and P, respectively) and the number of days without precipitation proceeding the sampling date (Dry Days) were also documented.

Spatial, temporal, and environmental analysis methods were utilized to determine the causation of bacterial outbreaks in wells. ArcGIS 10.4 was used for spatial analysis. Inverse distance weighted (IDW) interpolation using default settings was used to create temperature and precipitation maps of Alberta for each month that the study was active. The ArcMap Cluster and Outlier Analysis (Anselin Local Moran's I) tool was used to find statistically significant clusters and outliers of positive samples. This tool is meant to

be used on datasets containing weighted values, which is not the case with the binary fecal coliform data collected in this study.

Statistical analysis was performed in IBM SPSS. The methods of testing in SPSS were correlation analysis, multinomial logistic regression, and Bayesian regression. The MATLAB Classification Learner App was used to create classification trees using Gini's diversity index to decide when to split a node (Mathworks 2019). Multinomial logistic regression was performed using the following well characteristics: elevation, water removal flow rate, static water level, and total depth drilled.

Task 6.1. Provide an overall assessment to policy makers about potential risks associated with private well water systems as well as potential mitigation strategies for human, animal and environmental health.

Task 6.2. Provide recommendations regarding water testing frequency and microorganisms tested, livestock biosecurity and management and public perception management between agricultural productivity/economy and public health protection.

This project is not yet fully completed. Funding from AAF is available until November 30th 2020. We intend on inviting all stakeholders to a final meeting where we will present the findings of this large collaborative research project. Reports and presentations will also be prepared for specific stakeholders working in policy. It is anticipated that these meetings will take place online in September/October 2020.

C. PROJECT RESULTS

Please provide a narrative describing the key results using the project's milestones as sub-headings.

- Describe the importance of the key results.
- Include a discussion of the project specific metrics and variances between expected and actual performance.

RESPOND BELOW

STEC study results

The results as presented will be reported in draft manuscript(s) that will be submitted for publication in peer reviewed journals

The first project milestone addressed in these results relates to the identification, isolation and mapping of STEC from water well samples. This milestone was addressed across three different objectives and four different tasks outlined in the project proposal.

- Task 1.1. Test all archived *E. coli* positive private well water samples for stx 1 and stx 2 using qPCR on stored repositories;
- Task 2.1. Routine prospective testing of wells for *E. coli*/total coliforms and testing *E. coli* positive wells for STEC;
- Task 3.1. Collect GIS information relevant to animal husbandry, land-use demographics, etc;
- Task 3.2. Data integration of GIS information and laboratory water quality and pathogen surveillance

During the study period, 95,675 non-municipal drinking water samples were submitted to APL Calgary. Of these submitted samples, 2,565 (2.7%) were determined to be *E. coli* positive via Colilert®, a positivity rate of 268.10 per 10 000 submitted non-municipal drinking water samples. Two thousand one hundred and ninety (2,190) of these *E. coli* positive Colilert® enriched drinking water samples (85%) were tested for *stx1* and *stx2* by qPCR analysis. Of these qPCR-tested samples, 1899 samples were included in the study (87% of archived samples and 74% of total *E. coli* positive Colilert® enriched drinking water samples). Samples that were tested for *E. coli* and total coliforms but could not be successfully linked back to APL submission information were not included in the study. Seven percent (7%) of the *E. coli* positive Colilert® enriched drinking water samples (141/1899) were considered prospective samples having been submitted after May 21, 2015 and were processed accordingly. One-hundred and fifty-two (152) of the *E. coli* positive Colilert® enriched drinking water samples included in the study were qPCR positive for *stx1*, *stx2*, or both genes resulting in an overall *stx* occurrence of 8% within included *E. coli* positive drinking water samples (152/1899) and an estimated *stx* positivity of 0.2% within all voluntarily submitted non-municipal drinking water samples (152/95,675). Of the 152 *stx* positive Colilert® samples, 54 were *stx1* positive (35.5%), 53 were *stx2* positive (34.9%), and 45 were both *stx1* and *stx2* positive (29.6%).

Eight-hundred and fifty presumptive STEC isolates were recovered and tested for the presence of *stx1* and *stx2* by colony qPCR to confirm the presence or absence of STEC-related toxin genes in the isolates. Two hundred and forty-nine (249) of the isolates were positive for *stx* via qPCR. These 249 isolates originated from 59 of the 152 (38.8%) *stx* positive Colilert® drinking water samples. Surprisingly, none of the isolates represented in the other 93 *stx* positive Colilert® drinking water samples were found to be *stx* positive, even though some samples were found to have high *stx* copy numbers per qPCR reaction during screening (i.e., >800,000 copies/5 µl), suggesting that a significant proportion of environmentally-derived STEC may not grow well on CA-STEC plates. Accordingly, of the 249 isolates, 176 isolates were *stx1* positive (70.7%), 57 isolates *stx2* positive (23.7%), and 14 isolates *stx1* and *stx2* positive (5.6%). These 249 *stx* positive mauve isolates represented 57 STEC positive drinking water samples, with 36 of these samples positive for *stx1* (64.9%), 16 for *stx2* (29.8%), and 5 for *stx1* and *stx2* (8.8%).

The results obtained from the CA-STEC isolation revealed that in some drinking water samples multiple STEC isolates were present, raising the possibility that more than one STEC strain might also be present in the same water sample. Of the 249 *stx* positive mauve isolates collected, 231 isolates underwent (GTG)5 rep-PCR and were subsequently 'fingerprinted' by high-resolution capillary electrophoresis DNA-fragment analysis using the QIAxcel® Advanced system. Six *stx* positive water samples, from which 18 STEC colonies were isolated, were not included in the (GTG)5 PCR analysis due to a laboratory error. Upon comparison of (GTG)5 fingerprints between all isolates from each respective *stx* positive water sample, 65 isolates were determined to be unique, representing 51 *stx* positive water samples. Some water sources contained only a single identifiable STEC clone even though multiple colonies were isolated from the sample (Solid selection – Figure 2), whereas other water samples were shown to be contaminated with several genetically unique strains of STEC (Dashed selection – Figure 2).

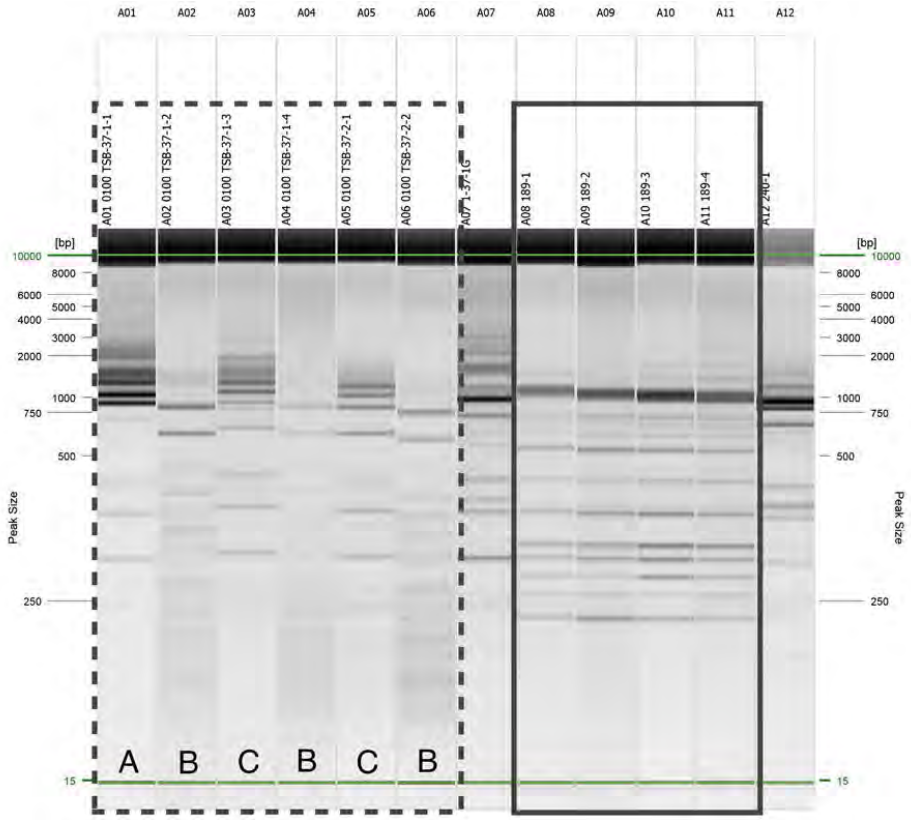

Figure 2: An example of a virtual gel image of the high-resolution capillary electrophoresis DNA-fragment analysis (QIAxcel® Advanced system) of (GTG)5 PCR products from 12 mauve CHROMagar™ STEC colonies isolated from 4 stx positive Colilert® enriched drinking water samples. The dashed selection highlights the DNA-fragment fingerprints of six stx *positive mauve colonies isolated from Sample #9100. Three unique (GTG)5 fingerprints (strains) are represented within these six isolates and are labeled (A,B,C) respectively. The solid outlined selection highlights the DNA-fragment fingerprints of four* stx *positive mauve colonies isolated from Sample #189. All four isolates share the same (GTG)5 fingerprint and represent a single STEC strain.*

Twenty-one STEC serotypes identified by the Canadian National Microbiology Laboratory were identified, the frequencies of which are outlined in Table 2. Five drinking water samples were shown to be contaminated with multiple serotypes (Table 3). Four of the 'big-six' non-O157 serotypes [O26, O103, O121, and O145,] (Table 2) were the most frequently detected serotypes in water, detected in 21 of the 51 STEC positive water samples (41%), with one sample containing both O26 and O145 serotypes (Sample #1677, Table 3). Of additional concern was the observation that several drinking water samples contained multiple STEC serotypes (Table 3).

Table 2: The frequency of STEC serotypes recovered from drinking water samples

Serotype	Number of Samples	Number of Strains
O145:NMa	6	7
O168:H8	6	7
O26:H11a	6	7
O121:H19a	5	5
O5:NM	4	4
O84:NM	4	4
O109:NM	3	3
O156:H25	3	4
O177:NM	3	4
O103:H25 a	2	2
O126:H8	2	4
O157:H7b	2	2
O26:NMa	2	2
O10:NM	1	1
O136:H12	1	1
O146:H21	1	1
O182 (O109):H25**c**	1	1
O182:H25	1	1
O183:H18	1	1
O46:H38	1	1
O8:H19	1	1

a Serotypes belonging to the non-O157 'big-six' clinical serotypes.
b Serotype commonly associated with clinically-relevant EHEC.
c A reaction with the O182 antisera, and a weak reaction with the O109 antisera

Table 3: Drinking water samples that contained multiple STEC serotypes and the corresponding STEC serotypes recovered.

Sample ID	Serotypes
241	O84:NM ; O177:NM
284	O145:NM ; O109:NM
332	O26:H11 ; O5:NM
1072	O8:H19 ; O126:H8
1677	O26:NM ; O145:NM

The highest annual positivity rate per 10 000 submitted drinking water samples was in 2005 (49.3) and the lowest annual positivity rate for a full year of submitted samples was 2010 (4.4) (Table 4). The statistical significance of the likelihood-ratio chi-square test that the dispersion parameter alpha (0.81) is equal to zero (75.9; $p < 0.001$) suggests that the response variable is over-dispersed and is best described by a negative binomial distribution rather than a Poisson distribution. The likelihood ratio test for this model suggests that year had an effect on *stx* positivity in submitted drinking water samples and account

for more variation in positivity than chance alone ($p < 0.05$). The year 2005 had a significantly higher *stx* positivity rate within submitted drinking water samples than the reference year of 2010, with the 2005 *stx* positivity rate in submitted drinking water samples 8.1 times the annual *stx* positivity rate in 2010 ($p < 0.05$). This in an important observation since this year was characterized by significant rain and flooding events in southern Alberta. Among the 13 years of data (2004-2016), annual prevalence of *stx* positive groundwater samples ranged from 4.35 – 49.26 per 10 000 submitted drinking water samples. Across the aggregate dataset, the mean prevalence of *stx* contaminated wells was 12 per 10 000 submitted samples (or 120 per 100,000 samples).

Table 4: Annual stx positivity rates per 10 000 submitted drinking water samples and negative binomial regression model of annual stx positivity rates in all non-municipal drinking water samples submitted to ProvLab Calgary compared to reference year 2010, March 2004 – July 2016

Year[c]	***stx* Positive Samples**	**Submitted Samples**	***stx* Positivity Rate (per 10 000)**	**IRR**	**Wald Test p-value**	**95% CI**
2004a	5	8548	5.85a	-	-	-
2005	70	14211	49.26b	8.39*	0.00	2.12 - 33.1
2006	10	9915	10.09	2.30	0.27	0.52 - 10.18
2007	8	9355	8.55	2.00	0.37	0.44 - 9.16
2008	9	7583	11.87	2.78	0.18	0.62 - 12.47
2009	4	7199	5.56	1.31	0.75	0.25 - 6.95
2010	3	6896	4.35	Referent	-	-
2011	9	6590	13.66	3.20	0.13	0.71 - 14.39
2012	4	6055	6.61	1.63	0.57	0.31 - 8.69
2013	10	6370	15.70	3.48	0.10	0.78 - 15.45
2014	5	5026	9.95	2.39	0.29	0.48 - 12.00
2015	4	5014	7.98	1.91	0.45	0.36 - 10.12
2016a	2	2913	6.87a	-	-	-

a Submission data incomplete for the full year
b Significant outlier (> upper fence of corresponding boxplot)
c Likelihood ratio Chi2 (12) = 22.13 ; p = 0.036
* Indicates statistical significance, $p < 0.05$

A monthly breakdown of STEC positivity was also analyzed from the aggregate dataset (Table 5). The lowest monthly positivity rate per 10 000 submitted drinking water samples was February (0.0), without a single *stx* positive sample from the over 4000 submitted drinking water samples from that month. Negative binomial and Poisson regression models struggle to calculate accurate IRRs for values equal to zero, therefore statistical significance could not be ascribed to the February IRR for monthly analyses and this month was not included in comparisons. The statistical significance of the likelihood-ratio chi-square test that the dispersion parameter alpha (0.61) is equal to zero (68.9; $p < 0.001$) suggests that the response

variable is over-dispersed and is best described by a negative binomial distribution rather than a Poisson distribution. The likelihood ratio test for this model suggests that month had an effect on *stx* positivity for submitted drinking water samples and account for more variation in positivity than chance alone ($p < 0.05$). Based on the standardized STEC occurrences per 1,000 *E. coli* contaminated water samples STEC occurrence was shown to be bimodal, with two dominant peaks occurring in early spring (i.e., March) and early summer (June/July).

Table 5: Monthly stx positivity rates per 10 000 submitted drinking water samples and negative binomial regression model of monthly stx positivity rates in all non-municipal drinking water samples submitted

Month[c]	***stx* Positive Samples**	**Submitted Samples**	***stx* Positivity Rate (per 10 000)**	**IRR**	**Wald Test p-value**	**95% CI**
Jan	2	5319	3.76	2.96	0.50	0.19 - 28.03
Feb	0	4862	0.00	< 0.01	0.99	0.00 - ∞
Mar	7	6439	10.87	7.75	0.08	0.78 - 61.92
Apr	5	6833	7.32	5.20	0.19	0.49 - 42.68
May	6	9503	6.31	4.47	0.22	0.43 - 36.18
Jun	64	11685	54.77	27.07*	0.00	3.20 - 203.81
July	29	12767	22.71	12.81*	0.02	1.47 - 97.46
Aug	13	10881	11.95	8.10	0.07	0.87 - 63.05
Sept	9	8869	10.15	6.31	0.12	0.65 - 49.89
Oct	4	7446	5.37	3.91	0.30	0.34 - 32.92
Nov	1	6385	1.57	Referent	-	-
Dec	3	4686	6.40	4.93	0.24	0.39 - 43.20

c Likelihood ratio Chi^2 (11) = 45.68 ; $p < 0.000$

* Indicates statistical significance, $p < 0.05$

Figure 3a shows the geographic location of each of 1,607 submitted *E. coli* positive drinking water samples along with their respective stx positivity as determined by stx1/stx2 qPCR analysis. Figure 3b provides the geographic location of each sample from which viable STEC was recovered along with the corresponding serotype information.

Spatiotemporal clustering examined using the Kulldorff scan statistic identified a cluster of 160 samples with a radius of 34.53 km during the time frame of 2005/6/20 – 2005/7/3 (Figure 3c). The cluster contained 19 observed *stx* positives compared to an expected 4.78 *stx* positives and had a Test statistic of 12.86 and a P-value of 0.0000045. The serotypes observed in this cluster included O10:NM, 026:H11, O46:H38, O109:NM, O103:H25, O121:H19, O145:NM (3 isolates), O156:H25, and O168:H8, with 4 of these serotypes represented by the 'big-six'. The most likely space-time cluster determined by the space-time permutation model encompassed both the purely spatial and purely temporal clusters determined by

Bernoulli scan analysis, re-emphasizing the importance of the area in terms of its susceptibility to contamination.

The Bernoulli spatial scan statistic identified a purely spatial cluster of STEC occurrences in the western portion of southern Alberta when all data was included in the analysis. This cluster included six samples within a 6.58km radius and contained 5 *stx* positive samples compared to an expected 0.48 *stx* positive samples, with a relative risk of 10.85, a log likelihood ration of 10.12 and a P value of 0047. The STEC serotypes identified in this cluster included O145:NM (3 isolates), and O121:H19, both of which are considered part of the 'big-six' serotypes of STEC. When the 2005 data was excluded from the analysis (i.e., as an outlier) another spatial cluster was observed on the southeastern border of the province (Figure 3d).

There were nine of the 65 serotyped isolates found to contain antimicrobial resistant *E. coli*. The STEC isolates from these specific samples were screened using the NARMS panel. One sample had two STEC strains of the same serotype (O26:H11), but with different resistance patterns. One strain was susceptible to all antimicrobials in the panel, whereas the other was resistant to tetracycline and sulfisoxazole. In addition to the differences in (GTG)5 rep-PCR fingerprints, the differences in the antibiograms of these two isolates further confirmed that there were multiple strains of O26:H11 STEC that had contaminated this drinking water sample.

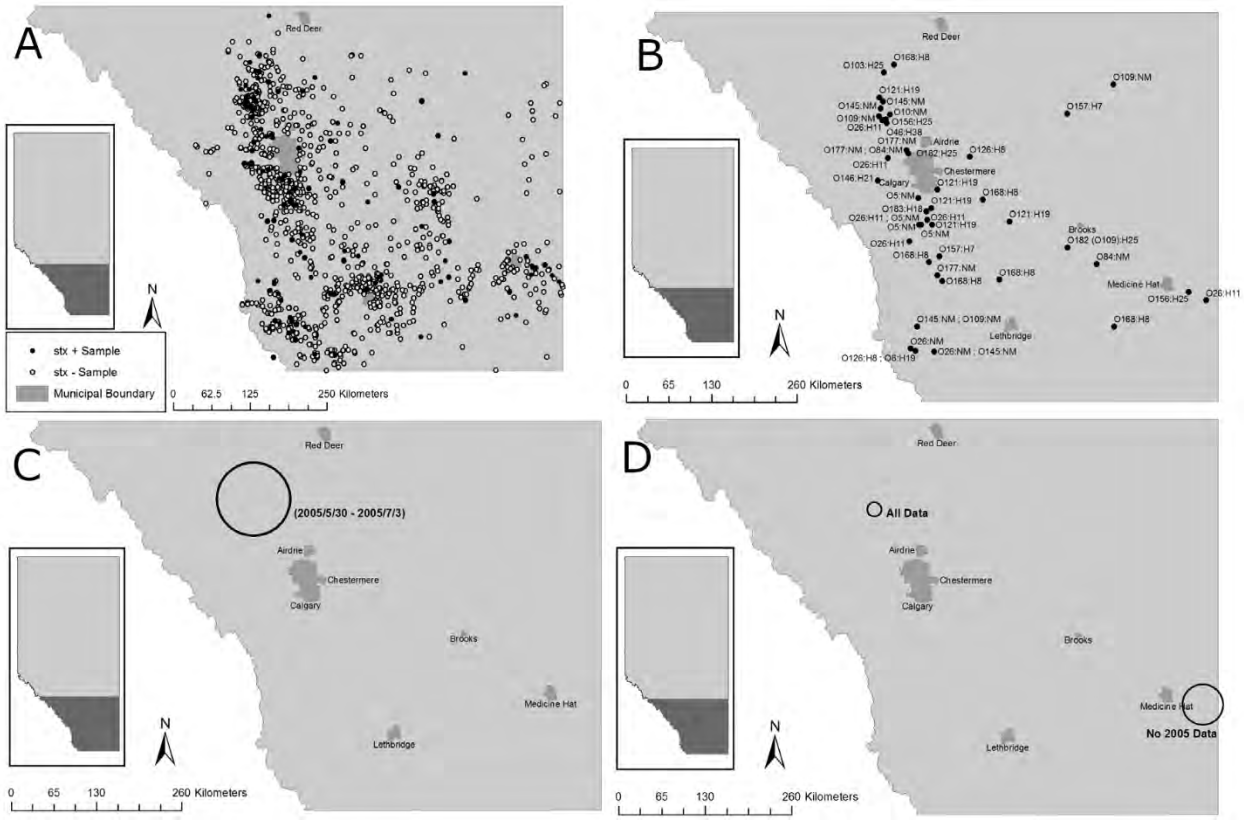

Figure 3: The geographic location of the corresponding water source for (A) All E coli *positive drinking water samples having complete ATS information during the study period. Light coloured dots represent* E.

coli *positive,* stx *negative, drinking water samples and dark coloured dots represent* E. coli *positive,* stx *positive, drinking water samples (B) each serotyped STEC recovered from drinking water samples (C) the most likely space-time cluster of* stx *positive water samples using a space-time permutation model Kulldorff scan (D) the most likely spatial cluster of* stx *positive water samples using a Bernoulli model Kulldorff scan for the total study period (circle representing 'All Data') and for a study period with 2005 data removed (circle representing 'No 2005 Data').*

STEC project specific metrics: The specific tasks set out for this sub-project were met in full. The project allowed for the training of a postgraduate student at the MSc level. To date, one publication has been published in the Canadian Journal of Microbiology (Reynolds et al., 2020), and two more draft publications are in the final stages of production. One MSc student and a research associate were trained in this area of the project.

AMR study results

The results as presented will be reported in draft manuscript(s) that will be submitted for publication in peer-reviewed journals.

The second project milestone addressed in these results relates to the identification, isolation and mapping of AMR from water well samples. This milestone was addressed across three different objectives and four different tasks outlined in the project proposal.

- Task 1.3. Screening for antimicrobial resistant *E. coli* in retrospective study by the agar screen plate method.
- Task 2.4. Screening for antimicrobial resistant *E. coli* in prospective study by the agar screen plate method, and including extended spectrum beta lactamase producing *E. coli.*
- Task 3.1. Collect GIS information relevant to animal husbandry, land-use demographics, etc;
- Task 3.2. Data integration of GIS information and laboratory water quality and pathogen surveillance

Samples submitted to APL between August 2006 and August 2016 were utilised. There were 67,339 samples submitted during this time period, of which 1328 samples were positive for *E. coli* (1.97%). These samples addressed task 1.3. An additional 121 *E. coli* positive samples were collected prospectively, of which three were rejected because the date of collection was not interpretable. These samples addressed task 2.4.

Among the 1,328 *E. coli* positive samples, 1,129 were included in the study as samples were archived when time permitted. Thirty three percent of samples (374/1,129) has at least one presumptively resistant *E. coli* isolate, meaning the isolate grew on antimicrobial-supplemented media in the agar screen test. Among these, 118 did not meet the species identification criteria for *E. coli* based on API® testing. Twenty-two percent (248/1,129) of the samples tested demonstrated resistance to one or more antimicrobials. Among these samples were 285 resistant and nine intermediate isolates. Thirty-five samples had two distinct resistance profiles, and five samples had three distinct resistance profiles.

The highest resistance observed was to tetracyclines, sulfonamides and aminoglycosides, observed independently in 79, 52 and 48% of isolates respectively (Table 6). Resistance to penicillin and chloramphenicol classes was detected among 38 and 18% of isolates respectively. Resistance to cephalosporins and macrolides were 9.8 and 8.8% respectively. When expressed as a proportion of the

number of *E. coli* positive samples tested, 12 and 11% were positive for tetracycline resistant and sulfonamides and/or aminoglycosides resistant *E. coli* respectively. Resistance to penicillin and chloramphenicol were observed in 8.9 and 4.3% or samples tested. Resistance to quinolones, cephalosporins and macrolides was detected in 3.0, 2.5 and 2.1% of samples tested respectively.

Table 6: Number and percentage of isolates and E. coli positive water samples with resistance to each of eight classes of antimicrobials tested by NARMS Sensititre panels.

Antimicrobial	**Number of resistant isolates N (%)**	**Number of *E. coli* positive water samples containing resistant isolates N (%)**
Tetracycline	225 (79)	196 (17)
Sulfonamide	149 (52)	132 (12)
Aminoglycoside	139 (48)	125 (11)
Penicillin	109 (38)	100 (8.9)
Chloramphenicol	52 (18)	49 (4.3)
Quinolone	40 (14)	34 (3.0)
Cephalosporin	28 (9.8)	28 (2.5)
Macrolide	25 (8.8)	24 (2.1)

Resistance to three or more classes (multi class resistance) was observed in 48% of isolates, with six isolates resistant to every class tested. The most common resistance profile (60/285) was to tetracycline alone, followed by resistance to aminoglycosides tetracyclines and sulfonamides in 11% of isolates (Figure 4). Among the 27 possible ESBL-producing isolates, 22 were positive for AmpC production, one was negative for both AmpC and ESBL production, and four were positive for ESBL production. Among the four ESBL producers, two were from the B2 phylogenetic group and two were from group A. AmpC producers were from phylogenetic groups A (8 isolates), D (8 isolates) and B1 (6 isolates) (Figure 4).

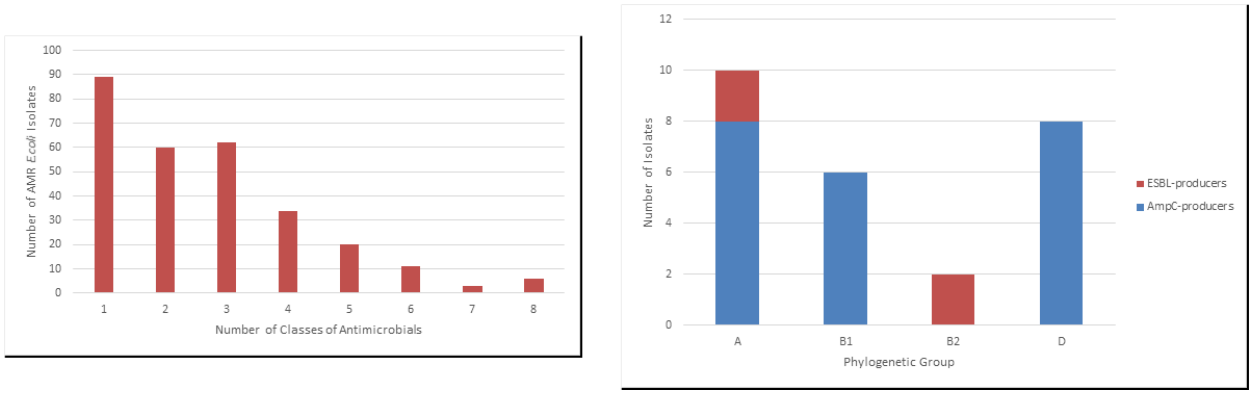

Figure 4: Number of classes of antimicrobials with resistance in each of 285 E. coli isolates isolated from rural well water sources (left) and a summary of the phylogenetic group results for ESBL and AmpC producing E. coli (right)

Geolocation: Of the 1,241 samples screened for resistance, 741 (59.7%) included location information. Of the samples that had locational information, 486 (65.6%) were single samples for that particular quarter section. 101 quarter sections had multiple samples collected over the study period, though it is unknown whether those multiple samples are from a single well or single samples from multiple wells within the quarter section. For quarter section with multiple samples, the maximum number of repeats was 9 and the average number of samples was 2.5.

Spatial Clustering: A statistically significant cluster of high AMR *E.coli* positives were detected between Calgary and Lethbridge, spanning 64 km with a total of 49 out of 126 wells being positive for AMR *E. coli* (Figure 5, Figure 6a). The relative risk of AMR *E. coli* contamination within these wells was 2.07 with a likelihood ratio (LLR) of 10.81 (p-value = 0.008).

A significant cluster of low proportions of wells positive for multiclass resistant *E. coli* spanned a large region north of Taber and Medicine Hat (Figure 6b) (p=0.037; LLR=8.79; relative risk (RR)=0.081). Among 93 samples tested in this area, only one sample was positive for MCR *E. coli* based on our testing. All three samples tested from a 9.2 km region southwest of Drumheller and northeast of Calgary tested positive for CHL resistance (p=0.026; LLR=9.5; RR=24.7) (Figure 6c). Similarly, a significant cluster of resistance to TET antimicrobials spanned a 65 km region between Lethbridge and Calgary, with 50 out of 150 samples testing positive for TET resistance (p=0.00016; LLR 12.4; RR=2.31) (Figure 6d).

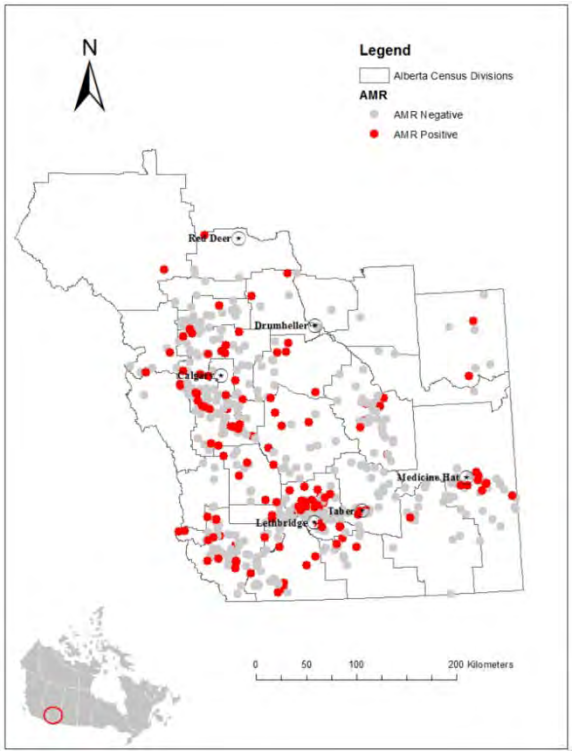

Figure 5: Antimicrobial resistance results for E. coli positive rural well water samples submitted to APL Calgary between 2006 and 2016 tested for AMR E. coli.

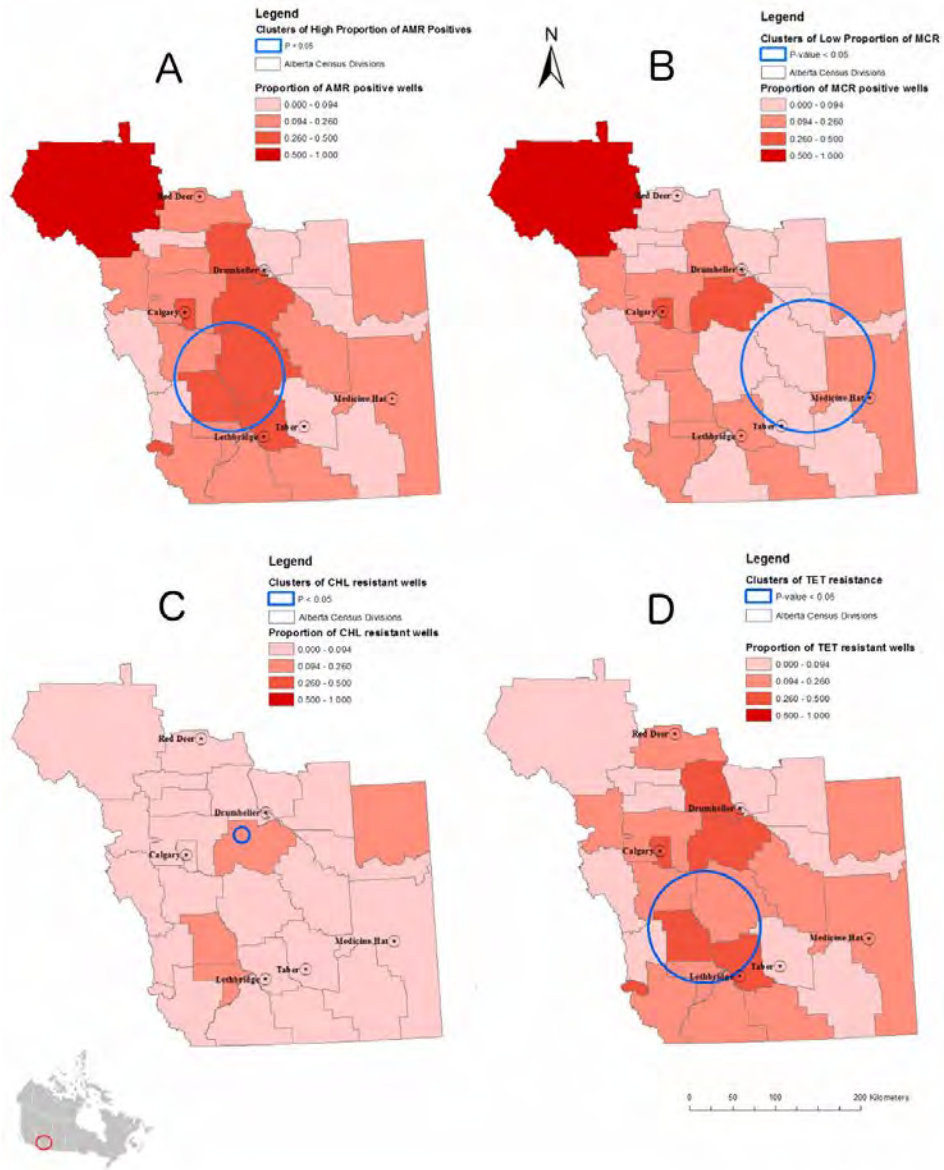

Figure 6: (A) Clusters of low proportions of MCR E. coli positive rural well water samples (B) Clusters of high proportions of AMR E. coli positive samples among E. coli positive rural well water (C) Clusters of rural well water samples positive for E. coli resistant to chloramphenicol antimicrobials. Results displayed as a proportion of E. coli positive water samples tested for AMR E. coli. (D) Clusters of rural well water samples positive for E. coli resistant to tetracycline antimicrobials. Results are displayed as a proportion of E. coli positive well water samples. All results are from samples submitted to APL Calgary between 2006 and 2016 and tested positive for antimicrobial resistant E. coli.

Temporal analysis: Edward's test of seasonality (Table 7) was significant for all variables, except for ESBL, though results for MAC, QNL, CHL, CEPH and ESBL should be treated with caution, as there were too few positives for accuracy. Peak dates ranged from July 17 to Jul 29 for variables with significant results.

Table 7: Edward's test of seasonality results.

Month	Tests Performed	AMR pos	MCR pos	MAC pos	CHL pos	QNL pos	CEPH pos	PCL pos	TET pos	SULF pos	AMG pos	ESBL pos
Jan	2477	2	1	0	0	0	0	0	2	1	1	0
Feb	2357	1	0	0	0	0	0	0	1	1	0	0
Mar	2924	3	3	1	1	0	1	3	3	2	2	1
Apr	2917	3	2	0	1	0	0	1	3	2	2	0
May	3322	14	10	0	2	2	2	5	12	10	9	1
Jun	3994	34	21	4	8	6	4	14	28	20	21	1
Jul	4624	33	12	2	6	3	1	16	29	14	15	0
Aug	3873	28	14	5	5	5	6	11	22	18	17	1
Sep	3686	11	4	0	1	1	2	4	8	5	5	0
Oct	3705	11	7	3	4	1	1	4	8	7	7	0
Nov	3125	5	3	0	2	0	1	2	4	4	2	0
Dec	2069	5	1	0	0	0	0	2	4	1	2	0
Total	39073	150	78	15	30	18	18	62	124	85	83	4
	Amplitude	106.4	112.4	136	125.5	147.7	129	119.3	103.8	106.7	114.9	141.6
	Peak angle	205	195.8	206.5	199.8	199.1	208	201.7	199.8	198.8	201	146.3
	Peak date	Jul-26	Jul-17	Jul-28	Jul-21	Jul-20	Jul-29	Jul-23	Jul-21	Jul-20	Jul-22	May-28
	Chi-square	72.52	39.65	10.97	16.32	21.09	11.25	33.84	57.82	41.78	46.41	2.93
	p-value	.000	.000	0.004	0.000	.000	0.004	.000	.000	.000	.000	.231
	Treat with caution (too few samples)			Yes	Yes	Yes	Yes					Yes

Seasonal Trend Loess (STL) decomposition analysis demonstrated seasonality in agreement with that demonstrated in Edward's test. While STL decomposition for some classes of antimicrobial resistance had single seasonal spikes (AMR, MCR, AMG, QNL, TET, PCL, and SULF), others had 2 or even 3 closely located spikes (MAC, CHL, CEPH), Trends visualized using seasonal decomposition were quite variable between the different classes of antimicrobial resistance. All had an initial peak beginning in 2006 and peaking in 2007. Most had one or more secondary, smaller peaks in the years 2011-2013. QNL had a second peak in

2013 that was larger than the initial peak. CHL and CEPH had peaks starting in 2014 trending upwards past the end of the timeseries (Figure 7).

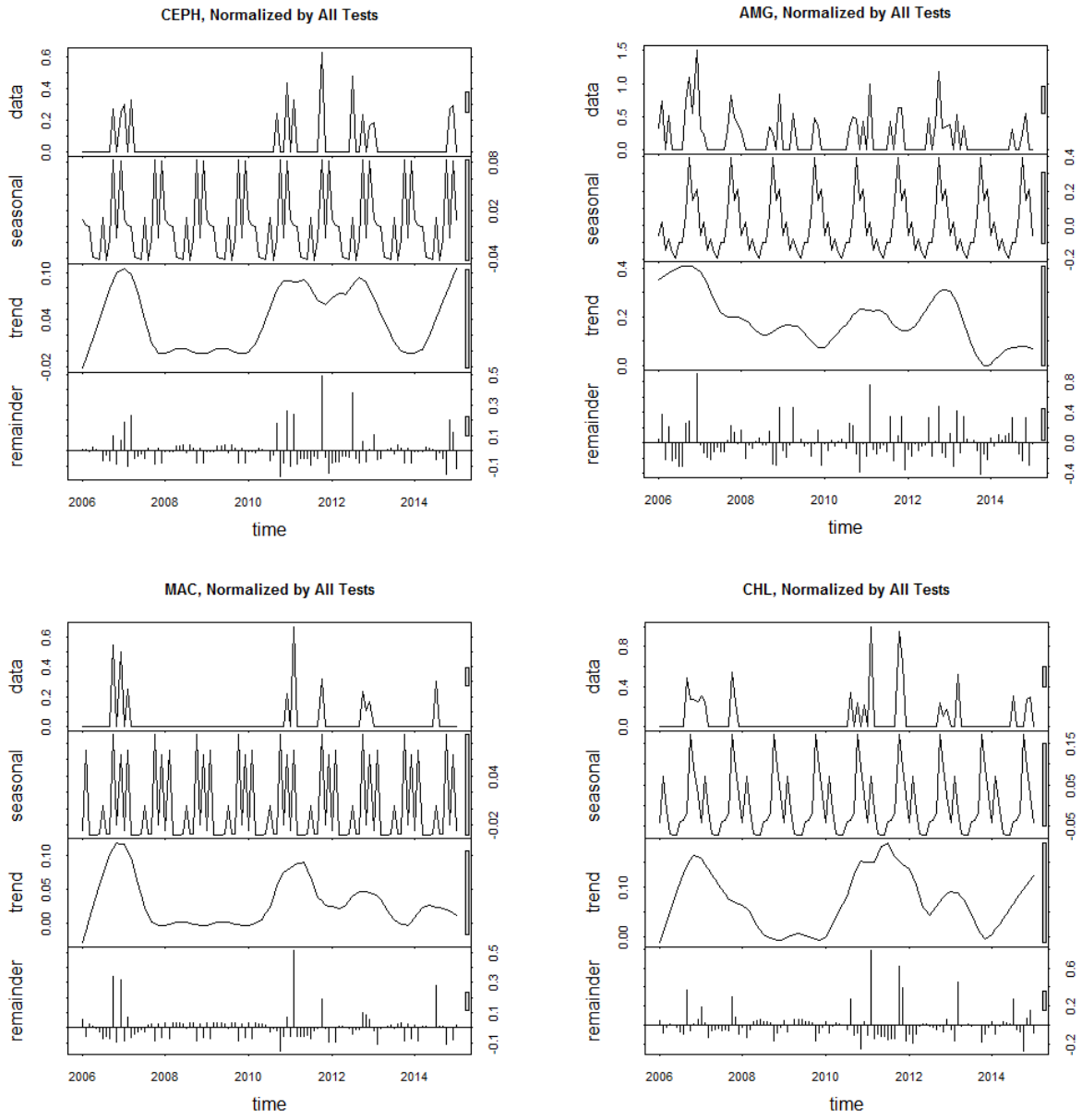

Figure 7: STL Decomposition output for selected analyses

AMR project specific metrics: The specific tasks set out for this sub-project were met in full. The project allowed for the training of a postgraduate student at the MSc level, and part-time employment for a research assistant. Two draft publications are in the final stages of production, one describing the AMR in the *E. coli* isolates and the other exploring the spatial and temporal patterns.

Recruitment study - questionnaire results:

Task 2.2 required the collection of private well water samples from livestock operations within the sentinel region. The results reported under this objective are as reported in Caffrey et al. (2020). Questionnaires were completed by 104 respondents, however six respondents did not use groundwater as their main source of drinking water, and on one property the groundwater distribution system was compromised. Therefore the results from these premises were removed from the survey, leaving 97 respondents.

Demographics: The respondents included 36 cow calf producers (37%), 14 feedlot producers (14%), 13 poultry producers (13%) and 34 acreage owners (35%). Acreage owners often reported keeping livestock, with cattle (73%) and poultry (18%) the most commonly reported livestock types kept. Of the respondents that indicated that they had an agricultural enterprise (n = 63), the median years farming was 30 years with a standard deviation of 13 years. Ninety-five and 92 respondents provided their age and education level, respectively. The education level, age and county in which respondents resided is provided (Figure 8). There was no statistically significant association among these factors indicating that the age (Fisher's exact P=0.5) and education level (Fisher's exact P=0.4) of the respondents did not vary significantly based on the location of the majority of the respondents. The distribution of respondents that were farmers versus acreage owners was also not significantly different.

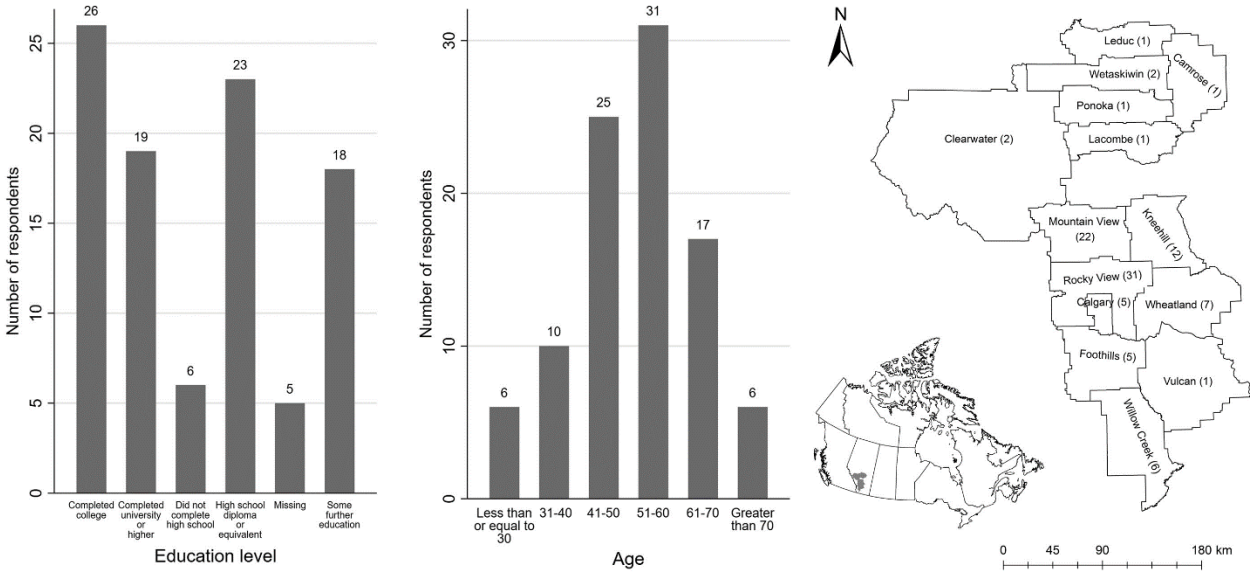

Figure 8: The education level, age and county of respondents

Bacterial test results: Bacterial test results were available for each participant. There were 20 TC+ tests, of which three were also *E. coli* (Colilert®) positive tests and two *Enterococcus* (Enterolert®) positive tests from 97 respondents (Table 8). Examination of the relationship between premises type and TC+ tests in an unconditional logistic regression model indicated that the type of premises is a borderline significant predictor of a TC+ bacterial test result with just a one-time sample (P = 0.07). The majority of tests were taken during fall (n = 37). Twenty-eight tests were taken in summer, 27 in spring, and five in winter. There was no statistical association between the season of testing and TC+ (P = 0.99).

Table 8: Bacterial test results in each premises type from the farm questionnaire

	Acreage	***Feedlot***	***Cow-calf***	***Broiler***
Total Coliforms (TC)				
Absent (TC-)	31	11	24	11
Present (TC+)	3	3	12	2
E. coli *(EC)*				
Absent (EC-)	34	14	33	13
Present (EC+)	0	0	3	0
Enterococci *(ET)*				
Absent (ET-)	33*	14	35	12
Present (ET+)	0	0	1	1
**Enterolert test for one acreage was not done*				

Water well design and construction: Water well drilling reports were available for 70 out of 97 premises (Table 9). Date of drilling ranged from 1961 to 2015. The majority of wells for which reports were available were completed using rotary equipment (n = 44), followed by cable tools (n = 16) and combinations (n = 8). Steel was most commonly used as a surface casing (n = 49, 78%), while plastic was the most common well casing (n = 47, 70%). The majority of annular seals were driven (n = 38, 57%), with a further 21% driven in combination with an application of bentonite or cuttings. There was no statistically significant unconditional association between the method of drilling (P = 0.3), the type of surface casing (P = 0.8), the type of well casing (P = 0.6) or the type of annular seal (P = 0.3) and the presence of TC+. There was no statistically significant unconditional associations when considering the well elevation, static water level, water removal rate or hydraulic resistance and the presence of TC+ (Table 9).

Table 9: Well construction characteristics from the drilling reports of 70 wells in the farm questionnaire

Variable:	Min	Median	Max	Mean	Odds Ratio	95% CI	P value
Well depth (meters)	12	41	97	43	1	0.98 – 1.005	0.3
Well age (years)	4	26	58	27	1.03	0.98 – 1.08	0.3
Elevation (meters)	256	326	1192	346	1	0.997 – 1.001	0.6
Water removal rate (liters per minute)	17	258	1290	288	1	0.99 – 1.01	1
Static water level (meters)	0.03	13	49	15	1	0.99 – 1.02	0.8
Hydraulic resistance (log_{10}[seconds])	7.9	10.7	11.6	10.5	0.8	0.36 – 1.62	0.5

Well location: Twenty-six wells were located inside a well pit (27%). The wells on 23 premises were located inside a pump house (24%). There was no unconditional association between a TC+ result and having the well in a pit (OR = 1.6, P = 0.4) or a well located inside a pump house (OR = 2.02, P = 0.2). The well was located in a serviceable location on 92 (94%) premises. On 12 premises, the well was located in an area

subject to flooding or ponding of water. There was no unconditional association between the location of the well (OR = 0.4, P = 0.3) or the presence of flooding or ponding of water around the well head (OR = 0.7, P = 0.7), and the TC+ result. Forty-five (46%) respondents indicated that records of the well operation and testing were available. Eleven premises indicated that there had been recent changes or repairs to their drinking water system. Eight premises had land use changes upslope of the well, including drilling or development of the land. Recent seismic activity was reported by six respondents. On eight premises, fuel tanks were reported upslope of the well. None of these factors were significantly related to the detection of total coliforms.

Presence of animals, manure and septic systems: Livestock were present within one km of the drinking water source on 86 premises. This included cattle (n = 82), pigs (n =2), broilers (n = 7), turkeys (n = 2), layer chickens (n = 13), or other types of livestock (n = 11). Where cattle were present there was no significant unconditional association with TC+ (OR= 1.8, P = 0.4). The presence of dogs (n = 66, OR = 0.6, P = 0.4), cats (n = 62, OR = 1.91, P = 0.2) or horses (n = 55, OR = 0.7, P = 0.5) were not associated with TC+ results. Wild animals such as deer were reported on 27 premises and wild birds were reported on seven premises. Neither were associated with TC+ test results. Where respondents indicated they owned an agricultural operation, 34 (63%) stored manure on their premises. There was no statistically significant association between the presence of manure on the premises and a TC+ test result (OR = 0.95, P = 0.9). The effect of storing manure on the premises was no different between cow/calf, feedlot, and poultry producers (Fisher's exact P = 0.6). A septic tank with leaching/drainage field was the most frequently reported (n = 65, 67%) type of septic system in use amongst respondents. Open discharge/ejector systems were utilized on six premises. Eleven respondents failed to respond to this question. Septic system age ranged from just one month to 100 years, with a median age of 18 years. On 27 premises, the respondent was unaware of the age of the septic system. Ten respondents failed to respond to this question. There was no association between the type of septic system and TC+ (Fisher's exact P = 0.5).

Setback distances from the main well: Respondents provided information about the distance from the well to structures including septic systems, manure, houses, barns, corrals and pastures on or near their premises (Table 10). Minimum set back distances from potential sources of contamination are outlined in the Nuisance and General Sanitation Regulations (Government of Alberta 2003). On 18 premises the minimum setback distance outlined for manure storage facilities, collection areas, or livestock yards was violated. However, in an unconditional association, there was no significant difference between the TC+ test results on premises that met and those that did not meet the setback distance (OR = 2.1, P = 0.5).

Table 10: The minimum setback distance required and the number of premises where the location of the well head violated the setback distance.

Area	Minimum setback distance (m)	Distance violated:	Odds Ratio	95% CI	P value
Manure storage facility, manure collection area, or livestock yard	100	18/47	1.04	0.2 – 5.0	0.9

Above-ground fuel tanks	50	3/52	0.3	0.05 – 2.3	0.3
Manure or composting materials application	30	Barns and Pens: <30m: 21/72	1.03	0.3 – 3.80	0.9
		Corrals and Pastures: <30m: 27/68	0.7	2 – 2.4	0.6
Watertight septic tank and field	15	4/63	-		0.6*
Pesticide fertilizer storage	30	2/26	-		1*
Existing buildings	3.25	10/91 houses[1]	1.06	0.2 – 5.5	0.9
		5/48 other buildings	-		1*
Outer boundary of a road or highway	6.1	2/65	-		1*
Garbage or landfill	450	Garbage: <450m: 12/31	1.3	0.2 – 8.7	0.8
		Landfill: <450m: 7/24	0.5	0.5 – 0.3	0.5

[1]The well was inside the home on three premises
*Fishers Exact test used

Water quality testing: Thirty-five (36%) respondents indicated that they regularly submitted samples for bacterial water testing, however, just 24 (25%) actually reported conducting bacterial testing at least annually. The remaining 11 respondents conducted testing every 2-3 years, or less frequently. Information on the frequency of bacterial testing, beyond that it was not done regularly, was unavailable on 63 premises. There was no unconditional association between the frequency of bacterial water testing and TC+ (OR = 0.9, P = 0.9). Poultry producers were significantly more likely to carry out annual bacterial testing than cow/calf producers (OR = 204, P = <0.001). Respondents reported conducting chemical testing either at least annually (n = 20, 21%), or having no schedule for chemical testing (n = 74, 75%). Three respondents did not provide any information relating to their chemical testing schedule. Similar to bacterial testing, there was a disparity between respondents that indicated regular testing and the frequency with which testing was reported. For example, six respondents that indicated testing was done regularly did not provide corresponding information when asked how frequently chemical testing was undertaken. Poultry producers were significantly more likely to conduct annual chemical testing on their water than cow/calf producers (OR = 57, P = <0.001) or acreage owners (OR= 19.3, P = <0.001).

Well maintenance: Thirty-five respondents (36%) never had their well shock chlorinated, while just two respondents indicated this task was done on an annual basis. Just 25 respondents indicated that their well had been shock chlorinated within the three years prior to completing the questionnaire. A further 13 respondents had shock chlorinated their well at some frequency beyond three years. The occurrence of the last shock chlorination was unknown by 19 respondents. The frequency of shock chlorination was not associated with the education level of the respondent (Fisher's exact P = 0.1). There was no unconditional association between having shock chlorinated the well within the past three years and TC+ test results

(OR = 0.95, 0.9). There was also no unconditional association between having shock chlorinated the well within the past three years and the premises type (P = 0.6). Feedlot operators most frequently reported having never shock chlorinated their well (71%). Acreage owners most frequently reported not knowing when the well was last shock chlorinated (32%). Cisterns were used for water storage on 25 premises (26%), with the number of cisterns in use ranging from one (14%) to six (1%). Cisterns were most often located indoors (n = 16, 16%). On seven premises the cistern was located underground. The use of cisterns was 9.9 times more likely amongst poultry producers (61%) than amongst cow/calf producers (14%) (P = 0.02). There was no association between the use of cisterns and a TC+ test in an unconditional logistic regression model (OR = 0.7, P = 0.5).

Well water treatment: Thirty-seven premises (38%) indicated the use of filtration on their primary water source, 16 indicated the use of disinfection, and 23 used a point-of-use device such as an on-tap filter or a jug filter. None of the 14 feedlots indicated any treatment of the water. Poultry producers were 8.4 times more likely than acreage owners to indicate that they treated their water (P = 0.05). The methods used to treat the primary water source were compared to those reported in the FWQS, (Fitzgerald, 2001) and the AWWS, (Summers, 2010) (Table 11).

Table 11: Methods of water treatment reported from three surveys

Method	***FWQS (1995-1996) (%) (n = 816 farms)**	****AWWS, 2010 (%) (n = 1,014 well owners)**	**Farm survey (%) (n = 97 well owners)**
Water softener	22	25	21
Iron filter	12	18	12[1]
Reverse Osmosis	3	13	13
Sediment filter[2]	NR	12	18[2]
Carbon filter	NR	8	7
Distillation	13	5	2
Constant chlorination	NR	3	11
UV system	NR	1	4
Other	3[3]	1	4

[1]Includes the use of greensand, [2] Includes use of dual multimedia, sand, and cartridge filters, [3]Some form of disinfection. NR = Not reported
*1995-1996 data from Farmstead Water Quality Survey (Fitzgerald, 2001)
**2010 data from Alberta Water Well Survey (Summers, 2010)

Respondents were asked to consider whether there was a current problem with their tap water, or if there had been a problem in the past 5 or 10 year period. In terms of current issues with their water, bad smell (n = 15) and bad taste (n = 12) were the most common issues identified by respondents

When respondents were asked if the quality of their tap water had changed over the previous 10 years, just four respondents were in agreement. Changes reported related to the smell, taste, colour and clarity of the drinking water. Sixty-nine (71%) respondents indicated they like to drink the tap water at their premises. Eighty-nine (92%) respondents indicate they never boil their water prior to consumption.

Three respondents who use their primary water source for drinking indicated they always boil their water prior to consumption. The remaining five respondents boil water for other purposes or rarely boil water prior to use. Reasons for boiling or treating tap water included to remove impurities (n =11), improve taste (n = 10), remove chemicals (n = 7), ensure safety (n = 9), treated water is healthier (n = 6), preference for filtered water (n = 8), habit (n = 1), and because they were told to (n = 3).

Fifty-one respondents indicated they purchase bottled water, with 15 respondents indicating that all of their drinking water was bottled, and 18 respondents indicating between 50 and 90% of their drinking water was bottled. Odds ratios of 0.25 and 0.06 indicate that cow/calf producers and poultry producers respectively were less likely to purchase bottled water than acreage owners (P = 0.008 and 0.001, respectively). When asked to consider how much of their drinking water was bottled five years ago and ten years ago, nine and six respondents indicated 100% respectively.

Twenty-eight respondents were concerned that their water source would become contaminated. Acreage owners were 2.7 times, 5.3 times, and 10.7 times more likely to report concern about well water contamination than cow/calf producers (P = 0.06), feedlot operators (P = 0.05), and poultry producers (P = 0.03) respectively. Respondents who were concerned that their well water will become contaminated were no more likely to conduct annual bacterial testing (OR = 1.02, P = 0.97), chemical testing (OR = 0.8, P = 0.7) or shock chlorination within three years (OR = 1.2, P = 0.7) than those who did not worry about water contamination. There was no significant association between purchasing of bottled water and respondents who indicated they worry their water will become contaminated (OR = 2.05, P = 0.13). There was a tendency towards poultry farmers indicating that they are not concerned about their well water becoming contaminated (OR = 0.17, P = 0.1) when those 13 responses were compared with the other premises types combined (n = 84).

Seventeen respondents indicated that they were concerned that they might run out of water. Of these, 12 indicated that this affects their water consumption. Eight respondents indicated that they were aware of some issues that may have affected the quality of their water in recent times. Sixteen respondents indicated that they were aware of changes in their groundwater table. One respondent indicated that they think someone had become sick from drinking their water. Persons of high risk (e.g. infants, elderly or immunocompromised) resided on 26 premises. There was no significant association between the use of bottled water (OR = 1.8, P = 0.23), or respondents concerned about their water source becoming contaminated (OR = 0.88, P = 0.8) and the presence of high risk persons in the household.

Recruitment study project metrics: While the recruitment and distribution of respondents to the questionnaire deviated from that proposed in the project application, the target recruitment number was exceeded. The results of the questionnaire were published in the Canadian Water Resources Journal (Caffrey et al. 2020) and other sub-projects utilised the participants recruited during this study. This sub-project employed one postdoctoral associate, two project coordinators, and two research assistants over the duration of the project.

Virology study results

Task 2.3. Choose a subset of 50 wells, stratified by depth of well (deep, shallow), to use for virus testing viruses once monthly for 12 months to assess occurrence and seasonality of water contamination.

Viral qPCR testing and cell culture with advanced ICC-qPCR testing of post-culture samples were completed in the fall of 2018. There were 577 samples entered into testing and analysis. In general, 16 samples were enteric viruses positive (2.8%) by viral qPCR testing, 11 positive (1.49%) by ICC-qPCR and one positive in cell culture with visible cytopathogenic effect (CPE) (Table 12). The results in cell culture and ICC-qPCR indicated that there was potential infectious virus presence in groundwater samples. The most commonly detected virus was reovirus, followed by adenovirus, rotavirus and JC virus. No seasonal distribution of detected viruses in well water, indicating that viral contamination of well water might be an occasional event.

In parallel testing of bacteria from the tap water samples from those wells, the results showed that 64 samples were total coliforms (TC) positive (11%), nine *E. coli* (EC) positive (1.5%) and two *Enterococcus* positive (0.3%) by a florescent testing method. TC and EC positive samples were mostly seen in the months of June to October. In parallel testing via qPCR the results showed that 225 samples were positive for *Enterococcus* DNA, of which two were positive for a virus via ICC PCR. There were 71 samples positive for *E. coli* DNA of which one sample was positive for a virus via ICC PCR. There were 150 samples positive for *Bacteroides*, none of which were positive for a virus via culture or ICC PCR.

A preliminary analysis showed that there is no difference among the viral positive detection rates of samples from wells in different categories of rural properties. None of wells showed both detected enteric viruses via ICC PCR or virus culture methods and TC/EC in the samples taken at the same time from tap water of the same premises, indicating that viral contaminations of well water may derive from different sources or routes.

To further analyse the potential effects of well characteristics, type of aquifer and lithology on viral contamination of groundwater, a team of experts in Alberta Environment and Parks were contacted. All well ID and locations were submitted to them and related information was mined and provided to us for analysis in March 2020. Correlation between well characteristics, on-site septic system and viral occurrence was analyzed the data obtained in this study. Among the well characteristics, well seal appeared to be a critical factor related to positive detection of enteric viruses in general. Distance between the well and on-site septic system seems a critical element for potential contamination of viruses in the well water. A manuscript is under preparation and expected to submit to the Journal of Water Research in fall of 2020

Table 12: Human enteric viruses and bacteria in well water in Alberta rural areas

Wells and samples			**Enteric viruses positive (numbers)**			**Bacterial positive (numbers)**		
User of well	No. well	No. Samples	q-PCR	ICC-qPCR	Cell culture	Total coliform	E. Coli	Enterococcus
Broiler farm	8	97	3 (3.1 %)			18 (18.6 %)	2 (2.1 %)	1 (1 %)
Cow/calf farm	12	145	5 (3.4 %)	2 (1.4 %)	1 (0.7 %)	16 (11 %)	1 (0.7 %)	2 (1.4 %)
Feedlot	8	95	1 (1%)	1(1%)	1 (1 %)	10 (10.8 %)	6 (6.4%)	0

Rural residents	20	240	7 (2.9 %)	7 (2.9%)		21 (8.7 %)		
Total	**48**	**577**	**16 (2.8 %)**	**11 (1.9 %)**	**2 (0.3 %)**	**65 (11.3 %)**	**9 (1.6 %)**	**3 (0.5 %)**

Task 2.5. Identification of repetitive well failures and perform extended pathogen testing (*Salmonella, Campylobacter, Cryptosporidium, Giardia*) – Dr Checkley and Dr Neumann

- Extended pathogen detection for the presence of Campylobacter and Salmonella using a molecular prescreen was carried out on all wells sampled in this subset.

The results as presented will be reported in draft manuscript(s) that will be submitted for publication in peer reviewed journals

The results of extended pathogen testing were analysed as part of two draft publications. The first used the results from the recruitment study in combination with the results from the prospective sampling of APL samples to compare molecular indicators of contamination with the standard methods currently in use. The second takes a more in depth look at persistent contamination and in particular six wells from which persistent contamination was detected over the course of the study. The results that will be presented in each publication are outlined below.

Comparison of molecular versus culture methods of identification of contamination: There were 549/42,269 routine samples positive for *E. coli* from the routine APL submissions over the duration of the study period. Of these, 202 were processed for molecular testing. Of the 264 available samples there were 45 identified by GIS co-ordinates to be samples from the same location. This ranged from two to five samples from 17 locations. Without further information relating to these samples, i.e., were they from the same well, or from the same point source the decision was made to remove these samples from further analysis. A further 17 samples without date information were also removed from the dataset. This left 202 *E. coli* positive samples from unique locations collected via routine submissions to APL.

Of the 764 samples submitted as part of the recruitment study, 694 samples from 97 premises were included in this study. The 70 samples removed did not represent groundwater (n = 58), had no molecular test results (n = 3), or were a second sample taken from a well in the same month (n = 9). The number of samples per premises ranged from 1 to 15. There were 35 premises with one sample only. Of this number, 87 samples (13%) were positive for total coliforms, 12 were positive for *E. coli*, and five were positive for *Enterococcus*. There were 607 water samples representing 82 premises that tested negative for both total coliforms and *E. coli*. From these negative samples the first sample taken from each well for which location information was available (n = 76) was used as a negative control for the 202 *E. coli* positive samples from the prospective sampling.

Frequency of detection of each water quality indicator: Bacteroides, *Enterococcus* and *E. coli* were detected by molecular methods in 66%, 63% and 21% of the 202 prospective study samples which were positive for culturable *E. coli*, respectively. *Arcobacter butzleri* was the most frequently observed pathogen in culture-positive *E. coli* groundwater samples, detected by qPCR in 9/202 samples (4%). *Salmonella* spp. and *Campylobacter* spp. were not detected in any of the water samples. In the recruitment study, 22%, 13%, and 5% of these *E. coli* negative samples were positive for the presence of

Enterococcus, *Bacteroides* and *E. coli* via qPCR respectively. *A. butzleri* was detected in one sample. *Campylobacter* spp. and *Salmonella* spp. were not detected in any of the 76 samples from the recruitment study.

When examining the strength of associations between the culture test and the molecular tests for detection of faecal contamination, the strongest association was between the presence of *E. coli* and *Bacteroides* (Pearson chi^2 = 61, P = <0.001, Kappa statistic = 0.4 (Moderate agreement)) (Table 13). There was also a strong association between *E. coli* culture and molecular *Enterococcus* (Pearson chi^2 = 36, P = <0.001, Kappa statistic = 0.3 (Fair agreement)), and molecular *E. coli* (Pearson chi^2 = 10, P = 0.002, Kappa statistic = 0.09, (Slight agreement)). The association between positive molecular tests was also evaluated. There was a significant association between molecular detection of *Enterococcus* and *Bacteroides* (Pearson chi^2 = 36, P = <0.001) (Table 13). Similarly, the association between molecular detection of *E. coli* and both *Enterococcus* and *Bacteroides* were significant.

Table 13: Pearson's chi^2 and Fisher's exact P values for correlations, and Kappa statistic agreement between detection of E. coli via Colilert with detection of indicators of faecal contamination via PCR, and different methods of PCR.

Test	**Pearson Chi^2 (1 df)**	**Probability**	**Fisher's exact**	**Agreement (%)**	**Expected agreement (%)**	**Kappa**	**Probability**	**Rating**
E. coli (culture) & *E. coli* (molecular)	10	0.002	0.001*	41	35	0.09	0.0009	Slight
E. coli (culture) & *Enterococcus* (molecular)	36	<0.001*	<0.001	67	51	0.3	<0.0001	Fair
E. coli (culture) & *Bacteroides* (molecular)	61	<0.001*	<0.001	71	51	0.4	<0.0001	Moderate
E. coli (culture) & *A. butzleri* (molecular)	2	0.2	0.2*	30	29	0.02	0.1	Slight
E. coli (molecular) & *Enterococcus* (molecular)	15	<0.001*	<0.001	58	49	0.17	0.0001	Slight

E. coli (molecular) & *Bacteroides* (molecular)	6	0.02*	0.02	68	50	0.36	<0.0001	Fair
Enterococcus (molecular) & *Bacteroides* (molecular)	36	<0.001*	<0.001	54	49	0.1	0.009	Slight
*Indicates whether the Pearson's Chi^2 or Fishers Exact was most appropriate								

The relationship between the proportion of *E. coli* culture positive samples that were also positive for each molecular test with the proportion of *E. coli* culture negative samples positive for each test was examined. There were significant differences for molecular detection of *E. coli*, *Enterococcus* and *Bacteroides* (Table 14). The odds ratio indicates that if a sample was positive for *E. coli* culture it was 4.7 times more likely to be positive for molecular *E. coli*. Similarly, a positive *E. coli* culture sample was 5.9 times more likely to be positive for molecular *Enterococcus*, and 13 times more likely to be positive for molecular *Bacteroides*.

Table 14: Case-control study design with E. coli positive samples as cases and molecular positive samples as the exposed population

202 *E. coli* positive cases, with 76 *E. coli* negative controls					
Test (*E. coli* culture versus :)	**Cases exposed Number (Proportion)**	**Controls exposed Number (Proportion)**	**Odds Ratio**	**95% Confidence Interval**	**Probability >Chi^2**
E. coli (molecular)	42 (0.21)	4 (0.05)	4.7	1.6 – 19	0.002
Enterococcus (molecular)	127 (0.63)	17 (0.22)	5.9	3.1 – 11.5	<0.0001
Bacteroides (molecular)	133 (0.66)	10 (0.13)	12.7	6 - 29	<0.0001
A. butzleri (molecular)	9 (0.04)	1 (0.01)	3.5	0.5 – 155	0.2

Effect of season: The proportion of samples taken in summer and fall in the two studies was significantly different, with more samples in the recruitment study during these seasons. The association between the detection of *E. coli* (culture), and season was evaluated in an unconditional logistic regression model (Table 15). An odds ratio of 0.3 (P = 0.001, 95% CI: 0.14 – 0.54) indicated that there was significantly less *E. coli* detected via culture in fall than in summer. Winter and spring did not differ significantly to summer. Season was a significant predictor of a positive *Enterococcus* (molecular) test, with an increased odds ratio of 2.9 (95% CI: 1.4 – 6.1) in fall compared with summer and controlling for whether the sample was from the prospective (*E. coli* culture positive) or recruitment study (*E. coli* culture negative). For *Bacteroides*,

there was a trend towards season as a significant predictor (P = 0.1), with an increased odds ratio of 1.9 in fall compared with summer. There was no significant association between season and *E. coli* (molecular) and *A. butzleri* (molecular) positive samples, when controlling for the sample type. For each molecular marker tested, there was a significantly decreased odds of detecting the marker in samples from the recruitment study compared with samples from the prospective study.

Table 15: The odds ratio and P value associated with multivariable logistic regression models for E. coli, Enterococcus, Bacteroides and A. Butzleri, controlling for the season the sample was collected, and the source of the sample (prospective or recruitment study)

	E. coli (culture)		***E. coli* (molecular)**		***Enterococcus* (molecular)**		***Bacteroides* (molecular)**		***A. Butzleri* (molecular)**	
	Odds ratio	**P value**	**Odds ratio**	**P value**	**Odds ratio**	**P value**	**Odds ratio**	**P value**	**Odds ratio**	**P value**
Season (baseline is summer)		0.001		0.6		0.02		0.24		0.7
Winter	1.1	0.9	0.9	0.8	0.6	0.4	1.2	0.7	1.4	0.8
Spring	0.7	0.2	1.5	0.3	1.3	0.4	0.8	0.6	2.0	0.3
Fall	0.3	0.001	1.6	0.3	2.9	0.005	1.9	0.1	0.6	0.7
Sample type (baseline is Prospective)										
Recruitment	-	-	0.2	0.003	0.1	<0.001	0.05	<0.001	0.3	0.3
Constant	4.2	<0.001	0.2	<0.001	1.4	0.08	1.8	0.004	0.03	<0.001

There were no significant differences in the proportion of samples from the two studies taken in winter or spring. In both summer and fall the proportion of samples taken in each season were significantly different, with more samples from the prospective study taken in summer (49% versus 32%, P = 0.01), and more samples from the recruitment study taken in fall (39% versus 17%, P = 0.0001). The number of tests positive in each season for *E. coli* (culture) and *E. coli*, *Enterococcus*, and *Bacteroides* (molecular) is depicted in Figure 2. There were just 10 samples positive for *A. butzleri*; four in summer, four in spring, one in fall and one in winter. A similar increasing trend in detection of both molecular *Enterococcus* and molecular *Bacteroides* is seen, with a peak in detection in summer. The same trend, though less pronounced is seen in molecular *E. coli* and *A. butzleri*.

The association between the detection of *E. coli* (culture), and season was evaluated in an unconditional logistic regression model (Table 5). An odds ratio of 0.3 (P = 0.001, 95% CI: 0.14 – 0.54) indicated that there was significantly less *E. coli* detected via culture in fall than in summer. Winter and spring did not differ significantly to summer.

Each molecular marker was considered in a multivariable logistic regression model with season and the study type as predictors of a positive molecular test (Table 16). Season was a significant predictor of a positive *Enterococcus* (molecular) test, with an increased odds ratio of 2.9 (95% CI: 1.4 – 6.1) in fall compared with summer and controlling for whether the sample was from the prospective (*E. coli* culture positive) or recruitment study (*E. coli* culture negative). There was no significant association between season and *Bacteroides* (molecular), *E. coli* (molecular) or *A. butzleri* (molecular) positive samples, when

controlling for the sample type. For each molecular marker tested (excluding *A. butzleri*), there was a significantly decreased odds of detecting the marker in samples from the recruitment study compared with samples from the prospective study.

Table 16: The odds ratio and P value associated with multivariable logistic regression models for E. coli, Enterococcus, Bacteroides and A. Butzleri, controlling for the season the sample was collected, and the source of the sample (prospective or recruitment study)

	***E. coli* (culture)**		***E. coli* (molecular)**		***Enterococcus* (molecular)**		***Bacteroides* (molecular)**		***A. Butzleri* (molecular)**	
	Odds ratio	**P value**	**Odds ratio**	**P value**	**Odds ratio**	**P value**	**Odds ratio**	**P value**	**Odds ratio**	**P value**
Season[a]		0.001		0.6		0.02		0.3		0.7
Winter	1.1	0.9	0.9	0.8	0.6	0.4	1.2	0.7	1.4	0.8
Spring	0.7	0.2	1.5	0.3	1.3	0.4	0.8	0.6	2.0	0.3
Fall	0.3	0.001	1.6	0.3	2.9	0.005	1.8	0.1	0.6	0.7
Sample[b]										
Recruitment	-	-	0.2	0.003	0.1	<0.001	0.07	<0.001	0.3	0.3
Constant	4.2	<0.001	0.2	<0.001	1.4	0.08	1.8	0.003	0.04	<0.001
[a] – baseline is summer; [b] – baseline is prospective										

Persistence of indicators of contamination:

To evaluate the persistence of contamination the samples collected via the recruitment study were utilized as this allowed for evaluation of multiple tests from the same well over time, not possible to the same extent through analysis of the routine samples submitted to APL as part of the prospective study. Of 694 samples, 420 (60.5%) were positive for at least one indicator of contamination (including total coliforms). Ignoring total coliforms, 375 (54%) samples were positive for at least one other indictor of contamination. There were 101, 111 and 58 samples positive for *Bacteroides* or *Enterococcus* or *E. coli* and at least one other indicator of contamination (excluding total coliforms) respectively. There were 85 (33%) samples positive for both *Enterococcus* and *Bacteroides* simultaneously.

One-way tabulations and decomposition of counts into, between and within components in panel data and calculation of transitional probabilities was performed for results representing *E. coli* culture, and *Enterococcus*, *Bacteroides* and *E. coli* via qPCR (Table 17). The overall part of the table summarizes results in terms of sample-months. For example, there were 682 sample months of data in which the *E. coli* culture result was negative (98%), and 12 sample months in which the result was positive (2%). The between column repeats the breakdown in terms of wells: 94 wells ever had an *E. coli* culture negative sample (97%) and 12 ever had a positive sample (12%). However, there were only 97 wells in the dataset, meaning that there were wells that sometimes had an *E. coli* culture positive and at other times were negative. The within percent column reports the fraction of time a well had the specified value for *E. coli*. For example, conditional on a well ever having an *E. coli* culture negative, 99% of samples from that well were negative. Similarly, conditional on a premises ever having an *E. coli* culture positive sample 31% of samples from that well were positive. These two numbers are a measure of the stability of the *E. coli* values, and *E. coli* culture negative results are more stable than positive results. The total within percent

of 91% is the normalized between weighted average of the within percent's, that is, (94 x 99 + 12 x 31)/106. It is a measure of the overall stability of the *E. coli* results. The number of wells ever showing any contamination with an individual marker was highest for *Enterococcus* (molecular) (n = 67), followed by *Bacteroides* (n = 61) and *E. coli* (molecular) (n = 49). These numbers are drastically higher than the number of wells that were ever positive for contamination via culture (n = 12).

In general, the results of the molecular tests were less stable than the *E. coli* culture tests indicating that the molecular indicators of contamination in wells changed from negative to positive and vice versa more often than *E. coli* via culture. To further evaluate this observation the transitional probabilities for each water quality indicator were assessed. For *E. coli* via culture, on each sample month 98% of the *E. coli* culture negative samples remained negative in the next month; the remaining 2% became positive. Although an *E. coli* culture negative sample had a 2% chance of becoming positive in each sample month, the *E. coli* culture positive samples had 100% chance of becoming (or returning to) negative, i.e. there were no wells with consecutive *E. coli* culture positive samples. In *Enterococcus* (molecular) for each sample month, 69% of the negative samples remained negative in the next month; the remaining 31% became positive. Although an *Enterococcus* molecular negative sample had a 31% chance of becoming positive in the next sample month, the *Enterococcus* molecular positive samples had 46% chance of becoming or returning to negative and a 54% chance of remaining positive. This indicates that a number of wells were consecutively positive for the presence of *Enterococcus* via qPCR. This result was similar though less pronounced for *Bacteroides* and *E. coli* molecular samples.

Table 17: Decomposition of counts into between and within components and transitional probabilities for each water quality indicator

	Decomposition of counts					**Transitional probabilities**			
		Overall Freq. (%)	**Between Freq. (%)**	**Within %**		***E. coli* (culture)**	**0**	**1**	**Total**
***E. coli* (culture)**	0	682 (98)	94 (97)	99		**0**	579 (98)	9 (2)	588
	1	12 (2)	12 (12)	31		**1**	9 (100)	0 (0)	9
	Total	694 (100)	106 (109)	92		**Total**	588 (98)	9 (2)	597
		Overall Freq. (%)	**Between Freq. (%)**	**Within %**		***Enterococcus* (molecular)**	**0**	**1**	**Total**
***Enterococcus* (molecular)**	0	434 (62)	86 (89)	74		**0**	263 (69)	116 (31)	379 (100)
	1	260 (38)	67 (69)	50		**1**	100 (46)	118 (54)	218 (100)
	Total	694 (100)	153 (158)	63		**Total**	363 (61)	234 (39)	597 (100)

		Overall Freq. (%)	Between Freq. (%)	Within %		*Bacteroides* (molecular)	0	1	Total
***Bacteroides* (molecular)**	0	522 (75)	88 (91)	84		**0**	339 (74)	118 (26)	457 (100)
	1	172 (25)	61 (63)	38		**1**	101 (72)	39 (28)	140 (100)
	Total	694 (100)	149 (154)	65		**Total**	440 (74)	157 (26)	597 (100)
		Overall Freq. (%)	**Between Freq. (%)**	**Within %**		***E. coli* (molecular)**	**0**	**1**	**Total**
***E. coli* (molecular)**	0	612 (88)	95 (98)	92		**0**	461 (88)	65 (12)	526 (100)
	1	82 (12)	49 (51)	20		**1**	62 (87)	9 (13)	71 (100)
	Total	694 (100)	144 (148)	67		**Total**	523 (88)	74 (12)	597 (100)

Investigating persistent contamination identified among a subset of wells

There were 694 water samples from 97 wells analysed as part of this study. The prevalence of indicators of contamination at the sample level is outlined (Table 18).

Table 18: Results of bacteriology and molecular pre-screen of 97 private water samples from rural Alberta

Indicator	Number positive (%)	Number negative (%)
TC	87 (13)	607 (87)
EC	12 (2)	682 (98)
*ET	5 (<1)	683 (99)
mEC	82 (12)	612 (88)
mET	260 (37)	434 (63)
mGB	172 (25)	522 (75)
mAR	15 (2)	677 (98)
mCA	1(<1)	691 (>99)
~mHF	3 (<1)	371 (99)
N = 694 *TC = Total Coliforms EC = E. coli ET = Enterococcus* *mET = molecular Enterococcus* *mEC = molecular E. coli* *mGB = molecular general Bacteroides*		

mAR = molecular Arcobacter Butzleri
mCA = molecular Campylobacter
mHF molecular Bacteroides HF183 marker
*Six samples did not have an Enterolert test
~Samples that were mGB+ were tested for HF183

The varying combinations of positive tests were evaluated. The results indicate that contamination of water samples was more frequently at the molecular level (n = 31 wells), or a mixture of molecular and standard positive tests (n = 42 wells) than detected via standard testing procedures (i.e. Colilert) (n = 7 wells). Where a well was positive for contamination via only standard testing procedures there was only one sample available for that well over the duration of the study period. There were 17 wells that had zero indicators of contamination. Of these 17, the majority (n = 13) were from wells that had just one sample. None of the 48 wells for which there was a minimum of 12 samples were free of all indicators of contamination over the entire study period.

The most common test result (mET+ only) occurred in 130 samples (19% of all tests) (Table 2). This was followed by mGB+ (9% of all samples), and the combination of mET+ and mGB+ (8% of all samples). A water sample that indicated contamination via a TC+ only occurred in 39 samples (6% of all samples).

The number of wells from which each test result combination was reported was also explored. There were 50 wells from which an mET+ only test was reported. Within these wells the range of samples positive was from one to seven. The most frequent combination of standard tests and molecular tests reported was a TC+ and mET+ concurrent result (n = 15). This combination accounted for just 2% of all positive tests.

Table 19: The prevalence of indicators of contamination including standard Colilert methodologies and detection via PCR

Test results in order of frequency (n = 694)	**Test result source**	**Freq. samples**	**% of total tests (n = 694)**	**Number of premises with this result (min – max sample-months)**
All negative	**Any test**	**274**	**39**	
mET only	PCR only	130	19	50 (1 – 7)
mGB only	PCR only	61	9	41 (1 – 3)
mET & mGB	PCR only	57	8	34 (1 – 5)
TC	Colilert only	39	6	23 (1 – 6)
mET & mEC	PCR only	20	3	14 (1 – 3)
mEC	PCR only	18	3	16 (1 – 2)
TC & mET	Mixed	15	2	14 (1 – 2)
mET & mEC & mGB	PCR only	14	2	13 (1 – 2)
mEC & mGB	PCR only	13	2	13 (1)

EC	Colilert only	6	<1	6 (1)
TC & mEC	Mixed	6	<1	6 (1)
TC & mET &mEC	Mixed	5	<1	5 (1)
TC & mGB	Mixed	5	<1	5 (1)
mGB & mAR	PCR only	5	<1	1 (5)
mAR	PCR only	4	<1	2 (1 – 3)
mET & mGB & mAR	PCR only	3	<1	1 (3)
mET & mGB & mHF	PCR only	3	<1	1 (3)
TC & mET & mEC & mGB	Mixed	2	<1	2 (1)
EC & ET & mET & mGB	Mixed	2	<1	2 (1)
EC & mET	Mixed	2	<1	2 (1)
TC & mEC & mGB	Mixed	2	<1	2 (1)
mET & mAR	PCR only	2	<1	2 (1)
EC & ET & mET & mEC	Mixed	1	<1	1
EC & mET & mGB	Mixed	1	<1	1
ET & mET & mGB	Mixed	1	<1	1
TC & mET & mGB	Mixed	1	<1	1
ET & mET & mGB & mCA	Mixed	1	<1	1
mEC & mGB & mAR	PCR only	1	<1	1

Evaluation of six most severe persistently contaminated wells: The transition probabilities for individual wells for which there were a minimum of 12 monthly samples and where at least 90% of samples were positive for at least one indicator of contamination were calculated (n = 6) (Table 19). In 5/6 wells, contamination with *Enterococcus* detected via qPCR was persistent, with wells *remaining positive* from 62.5% (sample 20020) to 90% (sample 20017 and 20013) of samples. For wells 20017 and 20013 persistent contamination was also detected via the *Bacteroides* marker (67% of samples). One well (20014) was positive for the presence of *Arcobacter Butzleri* in 100% of samples (n = 12). There was no total coliforms detected in that well throughout the sampling period. Well 47 had seven sample months in which the water sample tested positive for total coliforms and was the only well of the six that had an *E. coli* positive result via Colilert. That same well was also the only well of the 97 that tested positive for the presence of human faecal contamination via the HF183 *Bacteroides* marker.

Table 20: Transition probabilities for markers of contamination in each of six wells identified as having persistent contamination over the duration of the study.

Sample ID (num samples, % positive)	**Test**	**Number of months with a positive sample**	**Transition from negative to positive (%)**	**Transition from positive to negative (%)**	**Remain negative (%)**	**Remain positive (%)**
20017 –Acreage	TC	1	10	100	90	0
	mEC	3	33	100	67	0

(12 sample months, 100% positive)	mET	11	100	10	0	90
	mGB	4	25	33	75	67
20013 - Acreage (12 sample months, 100% positive)	TC	1		100		
	mEC	1		100		
	mET	11	100	10	0	90
	mGB	7	40	33	60	67
	mAR	1	10	100	90	0
20014 – Acreage (12 sample months, 100% positive)	mET	4	50	100	50	0
	mGB	8	100	57	0	43
	mAR	12				100
10090 –Acreage (13 sample months, 92% positive)	TC	4	50	12.5	87.5	50
	mEC	2	10	50	90	50
	mET	8	50	25	50	75
	mGB	5	43	60	57	40
	mAR	1	8	92		
47 – Broiler farm (13 sample months, 92% positive)	TC	7	60	43	40	57
	EC	1	9	100	91	0
	mEC	1	9	100	91	0
	mET	7	50	33	50	67
	mGB	4	25	50	75	50
	mHF	3	Missing results (only tested if GB+)			
20020 – Acreage (12 samples, 92% positive)	TC	1	10	100	90	0
	mEC	2	11	50	89	50
	mET	8	67	37.5	33	62.5
	mGB	2	22	100	78	0

GIS was used to map the location of the six wells in relation to factors of interest such as whether the well was located in a no till farming or irrigation area, and geological suitability for waste (Figure 9).

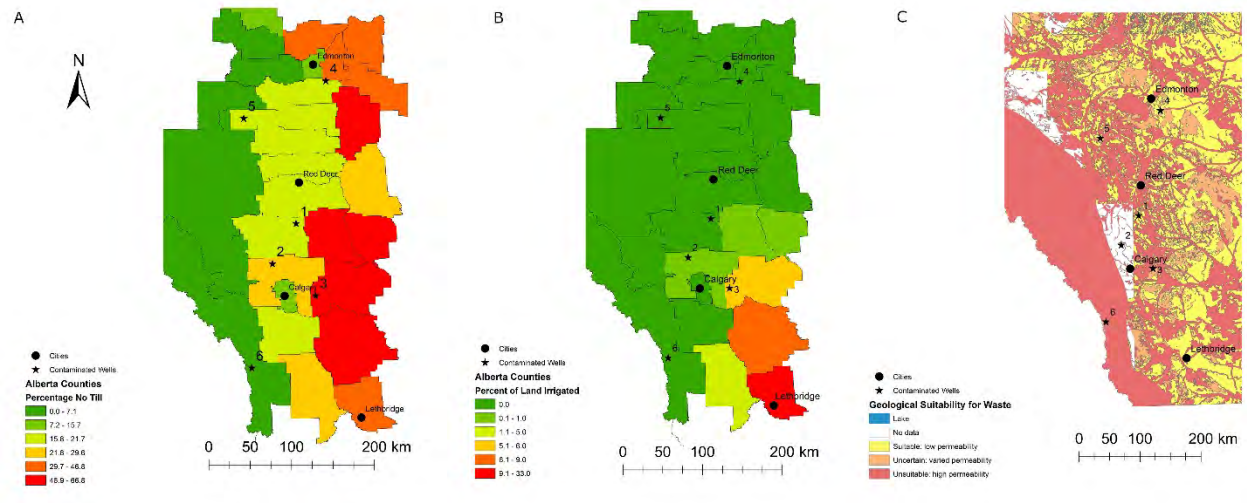

Figure 9: Percentage of land designated no till (A), Percentage of land irrigated (B) and geological suitability for waste (C) for each of six persistently contaminated wells idenified wells in this study.

The aim of **Objective 3** was to describe the temporal and spatial patterns of STEC and antimicrobial resistant organisms in well water across Alberta, both retrospectively and prospectively, and enteric viruses prospectively by assessing associations with environmental (climatic, geologic) and animal husbandry risk factors.

The results from the STEC and AMR work specific to this objective have been presented in the preceding sections.

The aim of **Objective 4** was to prospectively source track faecal contamination from *E. coli* positive wells within the sentinel region to assess epidemiological risk factors associated with contamination.

Of the 202 prospective samples utilized in objective 2, task 2.5 there were 133 samples from which *Bacteroides* DNA was identified. Of this number 129 were assessed for the presence of the HumM2 marker for human faeces and three were positive (2%). There were 128 samples assessed for the presence of the HF183 marker, of which 7 were positive (5%). There were 129 samples assessed for the presence of the ruminant marker CowM3, of which none were positive. These samples are described, however no statistical analyses was undertaken due to the low numbers.

On the three premises from which the HumM2 marker was identified, all three were positive for total coliforms and *E. coli* via culture and for *Enterococcus* and *E. coli* via qPCR. All three were also positive for the HF183 marker, and one was positive for *Arcobacter butzleri*. On the premises that were positive for the HF183 marker, there were four that were not positive for HumM2. Of these four, all were positive for total coliforms and *E. coli* via culture and *Enterococcus* via qPCR, and 3/4 were positive for *E. coli* via qPCR.

The aim of **Objective 5** was to examine livestock producers' perceptions of water quality and contamination and the influence of their perceptions on the management practices they choose related to mitigation of water contamination by cattle waste within the sentinel region.

Comparison of responses relating to water management from the recruitment study (farm survey) with the online questionnaire: A subset of 178 usable responses received as part of the online questionnaire were utilized to compare responses between the online questionnaire and the active sentinel site survey. One-hundred forty-four respondents (81%) indicated that they 'own or manage a farm, ranch, feedlot, or similar agricultural enterprise'. Of these, 122 (68.5%) identified as beef producers. Twenty-two respondents were a mixture of dairy (n = 1), layers (n = 1) mixed (n = 14), sheep and/or goats (n = 2) farmers, or did not respond (n = 4). Nineteen percent of respondents (n = 34) identified as acreage owners. Among the 160 respondents to the online questionnaire, the median number of years farming was 23 years with a standard deviation of 11 years.

The proportion of respondents that completed university was significantly higher in responses to the online questionnaire (n = 107) than the farm questionnaire (n = 19) (Z = 6.4, P = <0.0001). The proportion of respondents that completed high school or equivalent was significantly higher in respondents to the farm questionnaire (n = 23) compared with respondents to the online questionnaire (n = 6) (Z = -5.45, P = <0.0001)

In the online questionnaire, 95% (n = 168) of premises used their water for drinking, 97% of respondents used their water for household uses, and 12% of respondents used their water for agricultural purposes. While there was a trend towards respondents to the online questionnaire reporting that they drink their principal water source more frequently than respondents to the farm questionnaire, this was not significant (P = 0.1). The proportion of online questionnaire respondents using water for household purposes was significantly higher than for farm questionnaire respondents (P = 0.01). In contrast, the proportion of online questionnaire respondents using water for agricultural purposes was significantly lower than in farm questionnaire respondents (P = <0.0001).

Of the respondents to the farm questionnaire that indicated that they were an agricultural operation (n = 63), 37 (59%) stored manure on their premises. Of 155 respondents that answered the question regarding manure storage on the premises from the online study, 106 (68%) indicated that manure was stored on the premises.

In the online questionnaire, 92 (52%) respondents indicated that they have their well water tested for bacterial contamination. Just four respondents indicated a yearly frequency, 12 indicated it was done every 2-3 years and nine indicated it had been more than 3 years since the last test. The frequency of testing for the other 67 respondents was unknown. The proportion of respondents that indicated annual bacterial testing was significantly higher amongst the online questionnaire respondents (P = <0.0001). Just 17 respondents to the online questionnaire reported having regular chemical testing on their well water, with three respondents having this done at least annually. The other 14 respondents had no schedule for chemical testing. The proportion of respondents that indicated annual chemical testing was significantly higher amongst the farm questionnaire respondents (P = <0.0001).

In the online questionnaire, 152 (85%) respondents indicated if their well had ever been shock chlorinated. Of the 152, on 85 (56%) premises the treatment had taken place within the past three years. The last treatment was more than three years ago on six premises, and no information was given on 61 (40%) premises. The proportion of respondents indicating that they had shock chlorinated their well within the past three years was significantly higher in the online questionnaire (P = 0.0002).

In the online questionnaire, 134 (77%) of respondents reported having a cistern compared with just 26% of premises in the farm questionnaire. This is a statistically significant difference in the proportion of respondents reporting cistern use in each study type (P <0.0001).

In the online questionnaire, 161 (91%) respondents indicated that well water was not treated prior to use, in comparison to 62% of respondents to the farm questionnaire who indicated no use of filtration. The proportion of respondents that indicated some treatment of their well water was significantly higher amongst respondents to the farm questionnaire than the online questionnaire (P = <0.0001).

Sixty-nine (71%) respondents to the farm questionnaire indicated they like to drink the tap water at their premises. In the online questionnaire, 70 and 50 respondents (67%) agreed or strongly agreed that they are content with drinking water from their well. There was no significant difference in the proportion of respondents indicating that they like to drink or are content to drink their well water (P = 0.5).

In the farm questionnaire, 89 (92%) respondents indicate they never boil their water prior to consumption. In the online questionnaire, 47 and 51 respondents (55%) strongly disagreed or disagreed with the statement that they boil their water before drinking. There was a significant difference in the proportion of respondents boiling their water (P = <0.0001) between the two types of questionnaire respondents.

In the farm questionnaire, 51 respondents indicated they purchase bottled water, with 15 respondents indicating that all their drinking water was from bottles, and 18 respondents indicating between 50 and 90% of their drinking water was bottled. In the online questionnaire, 45 and six respondents agreed or strongly agreed that they purchase bottled water for drinking at home. The proportion of respondents indicating that they purchase bottled water was significantly higher in the farm questionnaire (P = 0.0001).

In the farm questionnaire, 28 respondents worried that their water source would become contaminated. In the online questionnaire, 12 and four respondents agreed or strongly agreed that they worry that their well water will become contaminated. There was a significantly higher proportion of respondents worried about well water contamination amongst respondents to the farm questionnaire than the online questionnaire (P = <0.0001). In the farm questionnaire, seventeen respondents indicated that they worry that they will run out of water. Of these, 12 indicated that this affects their water consumption.

In the farm questionnaire, 8 respondents indicated that they were aware of some issues that may have affected the quality of their water in recent times. Sixteen respondents indicated that they were aware of changes in their groundwater table. In the online questionnaire, 53 respondents agreed or strongly agreed with the statement that they were aware of contaminants that affect the quality of their drinking water. The proportion of respondents aware of contamination issues was significantly higher amongst respondents to the online questionnaire (P = <0.0001). In both questionnaires, one respondent indicated that they thought someone had become sick from drinking their water.

Qualitative Results: In depth, semi-structured interviews were conducted with 20 rural well owner participants (7 women, 13 men) for the qualitative section of this study. Participants ranged in age from 35 to 74 (mean 57). Summary statements (results) subsequent to thematic analysis of the semi-structured interviews under relevant Health Belief model categories are summarized schematically in Figure 10 and include the following:

Perceived susceptibility: there is very low risk of well water contamination; mitigation strategies are highly protective; well characteristics make contamination unlikely.

Perceived severity: although unlikely, if contamination were to occur it could have severe health effects on humans and livestock.

Perceived barriers: participants expressed difficulty in submitting samples during operating hours due to distance or participants' employment work hours, as well as the need to make a special trip. Participants felt the procedure was easy, feedback time suitable, and appreciated that there was no charge for the test.

Benefits of well water testing: well water testing was viewed as a diagnostic measure protective of human health, which also provides peace of mind that water is clean enough to drink; participants also noted this extended to benefitting livestock health, and the wider geographic community.

Cues to action: the no charge policy for water testing was viewed as supportive of increasing rates of well water testing in Alberta, although drop-off hours were seen as a barrier; some participants recognized it could be cost-prohibitive to expect the government to provide a pick-up service; mandatory testing would increase participation, though with increased public costs of enforcement; increased awareness of the water testing service was viewed as a strong option for increasing compliance.

Self-efficacy: participants felt water sample preparation was uncomplicated and a process they had completed.

Perceptions of drinking water quality: Most participants were comfortable with the quality of their drinking water and noted it tasted better than urban chlorinated water. Criticisms were the hardness of well water (12 participants) and sulphurous odour (4 participants).

Risks to well water contamination cited by participants included livestock and other agricultural activities, although viewing this as a risk factor was tempered by knowledge of responsible management practices, particularly where respondents owned livestock themselves. Livestock owners who were not engaging in "up to date" livestock and manure management practices were viewed as increasing risk of contamination of well water sources. Several participants also noted oil and gas exploration and excavation activities were a potential source of contamination, but felt industry was responsibly mitigating that risk.

Mitigation strategies noted by participants included shock chlorination, regular maintenance, and on site (farm or acreage) management strategies such as keeping livestock away from domestic well sources.

Participants were able to recall Walkerton as a major water contamination event in Canada. Ongoing debate between residents and various levels of government with respect to potential or realized water contamination in the communities of Beavermines, Harvey Heights, Edson, and Rimbey were cited.

Microbial contaminants (e.g., *E. coli*, Giardia) perceived to be sourced from drinking well water were mentioned by 17 participants as hazards to health with gastroenteritis as the clinical outcome. Pesticide and inorganic hazards were cited in general by 14 participants (e.g., pesticide runoff and nitrates) but few specifics were cited other than one case of potentially high fluoride content.

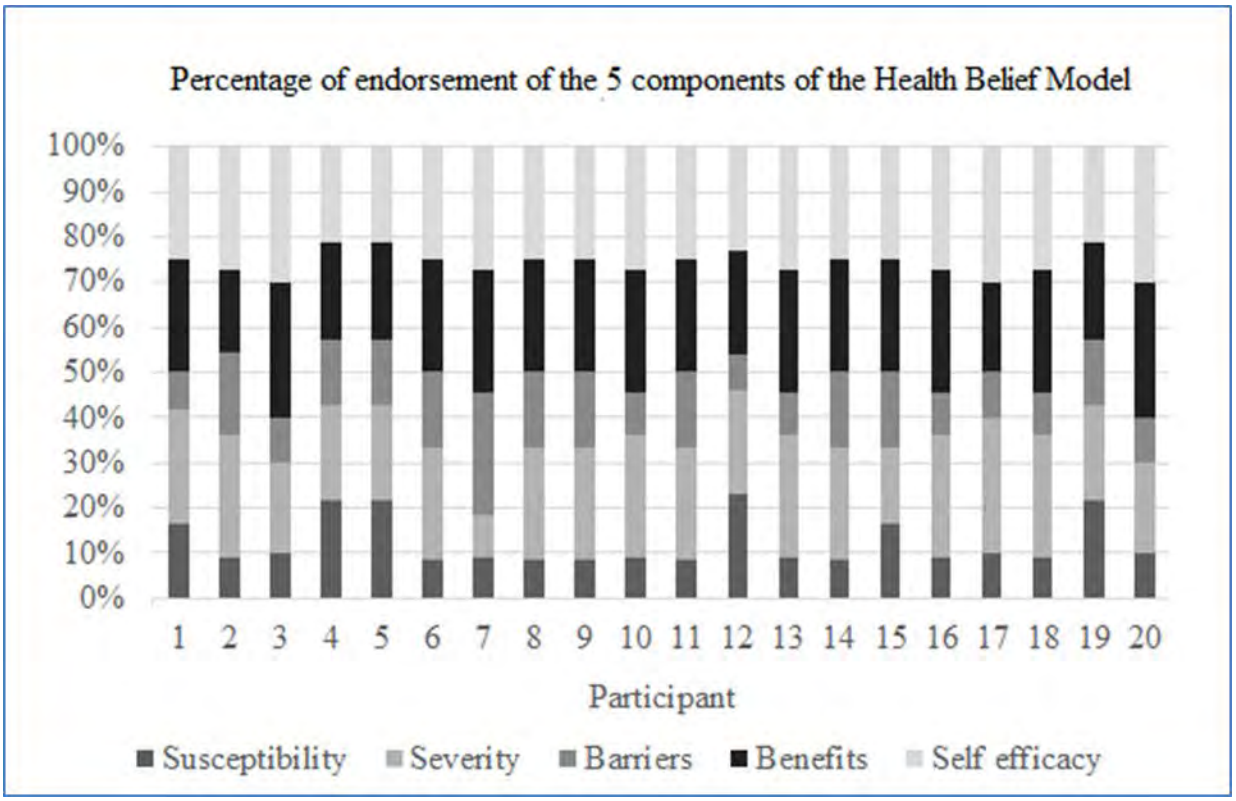

Figure 10: Participant endorsement of Health Belief model components based on questionnaire and semi-structured interview results.

A total of 23 well owners who self-reported livestock owners submitted drinking well water samples, none of which were positive for *E. coli*. Water was also collected from 28 rural participant sites (acreage owners) for presence of *Bacteroides* in drinking water (29 samples submitted) and a source of standing water near the drinking water well head (22 samples submitted). Standing water was located as close as 50m to 200-300m, rarely farther, from the well head. The *Bacteroides* results, as well as results of total coliform and E. coli tests run in our own lab, using the same methodology as described on page 12 are included in table 2. Twenty samples tested positive for *Bacteroides;* all positive samples came from surface water (*i.e.,* no household drinking water samples tested positive for *Bacteroides*). Not all general tests for *Bacteroides* corresponded with a particular species test. Of the 22 surface water samples, 20 tested positive for general *Bacteroides*, 7 tested positive for bovine *Bacteroides* and 4 tested positive for canine *Bacteroides*. There was 1 positive for human *Bacteroides* but no goose positives. Fourteen samples also tested positive for *E. coli*, only one of which was a drinking water sample from a well with a known problem, and 18 samples tested positive for total coliforms (two from drinking water samples).

The aim of **Objective 6** was to use information gained from the study to inform decision makers on the implications for human, animal and environmental health (e.g. water testing policies (microorganisms to test, lack of regulation of testing for private drinking water), risk maps, livestock biosecurity and other mitigation strategies).

The results reported from the sub-projects relating to objective 6 are outlined below:

Vulnerability mapping results

Groundwater vulnerability maps for the province, specific to *E. coli* in 2012 were generated in GIS using available soil geochemical properties (organic matter, pH), subsurface hydrogeological properties (hydraulic resistance, soil texture, soil moisture), and meteorological (precipitation) data. One key feature was the usage of province-wide precipitation and soil moisture data that enabled the estimation of seasonal groundwater vulnerability under both growing season and cold season conditions. The vulnerability maps gave an indication of where shallow aquifers could be intrinsically vulnerable to bacterial contamination. Final groundwater vulnerability maps showed the foothills region in western Alberta generally had the highest vulnerability, with lower vulnerability in the central and southern portions of the province (Figure 11). Vulnerability maps were tested against independent *E. coli* detection data from 2012, revealing that temporal factors (i.e., cold season soil moisture and growing season precipitation) had the greatest correlation with *E. coli* detections. However, the final maps lacked a statistically significant relation with *E. coli* detections, which is not surprising given the relatively infrequent detections. Overall, the results suggest the vulnerability maps should not be used for predictive purposes, but can be used for assessing variations in intrinsic groundwater vulnerability (Please refer to Van Staden et al., 2019).

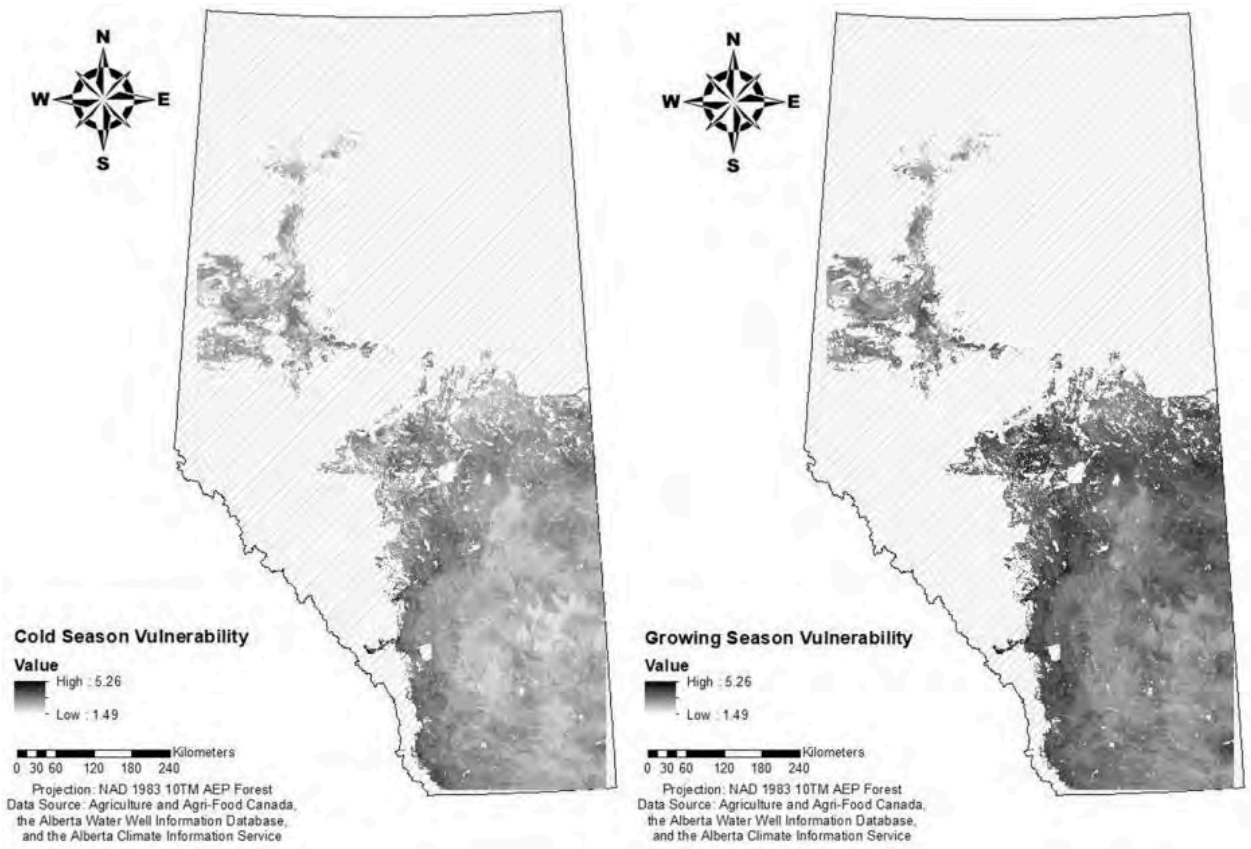

Figure 11: Cold season and growing season groundwater bacteria vulnerability maps for Alberta, 2012

VRAT study results:

The results as presented will be reported in draft manuscript(s) that will be submitted for publication in peer reviewed journals

There were 20 wells on which a direct threat VRAT outcome was identified and 13 wells where a potential threat was identified. Where the vulnerability was related to wellhead intrusion, 22 had well caps that were not rodent/insect proof (direct threat). There was a well with an annular seal that was not grouted (potential threat) and another two wells that did not have either a driven or grouted seals (direct threat). No wells failed the residence time benchmark, however, three wells had screens less than 15 metres (potential threat) and 10 wells had surface water within 100 (metres) potential threat. There were 14 wells with faecal point sources within established setback distances (septic fields and/or livestock yards). Human sewage was disposed into onsite subsurface disposal septic fields at all sites. No wells had lithology that would allow for quick passage of point source contaminants into the aquifer. Thirty-three wells had a water removal rate below 20 iGPM, with no sites with a water removal rate above 40 iGPM. Of the 40 sites, 21 (52.5%) tested positive for total coliforms, two tested positive for *E. coli*, and one tested positive for *Enterococcus* detected via standard Colilert and Enterolert. Thirty wells were positive for the presence of contamination detected via molecular methods at any sampling point at that well over the study period. There were six wells that were absent of any indicator of contamination. The presence of viruses was indicated at 13/26 sites. The overall agreement between the VRAT (no threat v potential/direct threat)

and the presence of a positive total coliform, *E. coli* or *Enterococcus* result was 45%. The overall agreement between the VRAT and a qPCR positive result was 70%. The overall agreement between VRAT and virology results was 46%.

Faucet study results: There were 11/21 samples from which some indicator of contamination was positive (2 via bacteria culture, 9 via qPCR) in samples taken prior to the disinfection procedure. There were 15/21 samples from which an indicator of contamination was identified after the disinfection procedure (2 via bacteria culture, 13 via qPCR). Sites with positive hits in this study generally depict a subset of organisms that were present throughout the overall study at each individual site. On 11 sites, a faucet in a different location was chosen for the 'optimal' sample, whereas 10 'optimal' samples were taken from the same location following disinfection of that faucet.

For the non-optimal sampling, 20/21 faucets had an aerator, 12 had a flex hose and none of the taps were leaking. One tap was rusted and corroded. The previous sampler did not remove the aerator for any of the taps. Three sites had a filtration system in place and three sites had a water treatment system. There were six sites that had water passing through both filters and a water treatment system. Two sites had intermediate cisterns that fed water to the house in addition to the well. Ten participants had shock chlorinated their well in the past. Seven of these participants shock chlorinated their well less than three years ago, one participant shocked their well 6 years ago, one participant shocked their well 8 years ago, and one participant shocked their well 15 years ago.

For the optimal sampling, all taps, except one, were used regularly. One tap had a flex hose. Three taps did not have an aerator. Of the 18 taps with an aerator, 16 had film or debris present on the aerator. Of the 18 taps with an aerator, the aerator could not be removed for five taps. Out of the 15 taps where the inside of the faucet was inspected, 6 taps had film or debris present inside the faucet. Staining was present on the water fixtures for three of the taps.

The proportion of samples positive before and after the disinfection procedure was not different statistically. The presence of the aerator, sampling location (kitchen or bathroom), presence of a flex hose, optimal versus non-optimal site and use of a camera to examine the interior of the faucet did not affect the presence of contamination.

Creation of a Tableau interface results: One of the goals for this project was to allow a user to explore demographics provided in the recruitment study questionnaire and to filter those to see how they impact the test results through Tableau. For instance, does having cattle or not having cattle on the premises impact the percentage of premises that are positive for *E. coli*? A series of interactive dashboards allowed users to visualize test results filtered by demographic information provided in the questionnaire. Multiple filters can be used within a story to focus on particular factors, for example, well management. On this dashboard the user can filter by five different variables simultaneously. The user can limit the display to a single premises type, such as cow/calf, or can limit based on questionnaire answers about water treatment and water testing. This Tableau interface allows exploration of questions such as "Are premises that regularly test for bacterial contamination more or less likely to perform shock chlorination, and what is the impact on the percentage of the premises that are positive for *E. coli*?" The antimicrobial resistance results can also be viewed on Tableau.

Results from modelling of rare event data:

The results as presented are those reported in the final report on this sub project (available in appendix 4).

Initial analyses of the microbial data included histograms created in Microsoft Excel, which created a visual representation of the temporal distribution and rarity of positive events. Both monthly and weekly histograms (April 2015 to December 2017) were created to describe samples obtained during the study. (Figure 12). Raster graphics displaying the monthly average temperature and precipitation for every month in April 2015 to December 2017 were created (Figure 13). Precipitation values are displayed in mm and temperature values are displayed in °C. There was a statistically significant cluster of total coliform positive wells revealed using cluster and outlier analysis, however most positive hits were statistically insignificant. For E. coli positive samples there was a single statistically significant well.

A full model including all environmental variables using multinomial logistic regression was investigated. Results indicated that this model did not perform well, with likelihood ratio tests of $p > 0.05$ for all independent variables. When examining the variables individually, T7 was the closest to being statistically significant. The result of the model indicated that increasing temperature by 1°C increased the likelihood of having bacterial growth by 2.8%. All logistic regression models using two or more variables to predict total coliform growth resulted in statistically insignificant model fits with $p > 0.1$ for all models. It is also noteworthy that all models predicted no positive coliform hits.

Logistic regression models were also created for *E. coli* and *Enterococcus* prediction. For *E. coli*, there were two models that performed quite well. The model using T7 and Max had a model fit of $p = 0.000$, a Pearson chi-squared significance of $p = 0.126$, and likelihood ratio tests of 0.000 for both Max and T7. The results indicate that if T7 is kept constant and Max temperature is increased by 1°C, the likelihood of *E. coli* growth is decreased by 26.4%, and if Max is kept constant then the likelihood of E. coli growth increases by 58.2%. This agrees with a previous study that found that higher temperatures were significantly associated with *E. coli* and fecal coliform levels (Wu et al., 2016; Cha et al., 2016).

A second model containing Max and T2 as predictor variables performed similarly, with a significant model fit of $p = 0.000$, a Pearson chi-squared significance of $p = 0.837$, and likelihood ratio tests of 0.000 for Max and T2. Similarly, this equation suggests that increasing the Max temperature results in a decreased likelihood of *E. coli* growth (by 37.1%) and increasing the two-day average temperature results in an increased likelihood of *E. coli* growth (by 71.9%). All models incorrectly predicted that there was no *E. coli* growth under any conditions.

Multinomial logistic regression performed on the well characteristics variables found all the individual variables to be statistically insignificant. Environmental factors evaluated using Bayesian linear regression models for every combination of the variables performed very poorly. Three separate models including only one variable each resulted in the same B10 value; these models are T2, T7, and T3. Each model had an equivalent posterior probability of 0.027, meaning there is a 2.7% chance of total coliform growth according to these models (note that 12.77% of samples were positive for total coliforms). The best Bayesian model for E. coli used Max and T3 and had $B10 = 0.805$ and a posterior probability of 0.053. The best Bayesian model for enterococcus included T3, T2, and T and had $B10 = 0.704$ and a posterior probability of 0.084. The next best performing model included T2 and T and had $B10 = 0.354$ and posterior probability = 0.042.

These models seem to agree with the multinomial logistic models that suggest temperature is the strongest indicator of bacterial growth. Notably, the best performing Bayesian regression model and

multinomial logistic regression model for total coliform presence both use T7. The Bayesian models did not include a prior distribution during this analysis, as none was available.

Machine learning methods including classification trees used to classify the data calculated accuracies of 83.8, 85.1 and 86.7 for fine, medium and coarse trees respectively. Note that predicting all responses to be 0 returns an accuracy of 87.3%, so the high accuracy does not necessarily indicate a model with useful information. 10-fold cross validation was selected to quantify the accuracy of each model. The fine tree, medium tree, and coarse tree models are defined as such by the maximum number of splits each tree can have: 100, 20, and 4, respectively.

The variables included on the trees can give some insight into important factors influencing the growth of bacteria (Figure 14). Only two variables appear on the coarse tree: the average precipitation and the minimum temperature on the sampling day. This suggests that the bacteria growth may be influenced by immediate events rather than weather conditions in days proceeding growth. The medium tree contains more predictor variables, such as Max, T2, T3, T7, P3, and P7. However, P2 and Dry Days still do not appear as predictor variables. By looking at the coarse tree model, high precipitation decreases the likelihood of total coliform growth. Total coliform growth can be seen when 6.15°C ≥ Min > 6.55 °C. This provides a very narrow range in which one can expect to have contamination. From the medium tree, total coliform growth is seen at low average precipitation (1.22 mm ≥ P7 > 1.38571 mm) and high average temperatures for preceding days (T7 ≥ 14.86 °C) when the sample date experiences low precipitation. For high precipitation days, the model suggests that lower temperature preceding the sample date (T2 < 12.425 °C) and lower max temperature (Max < 16.6 °C) is favourable for coliform growth. This seems to partially agree with the results from logistic regression; high weekly average temperatures and lower max temperatures seem to support bacterial growth.

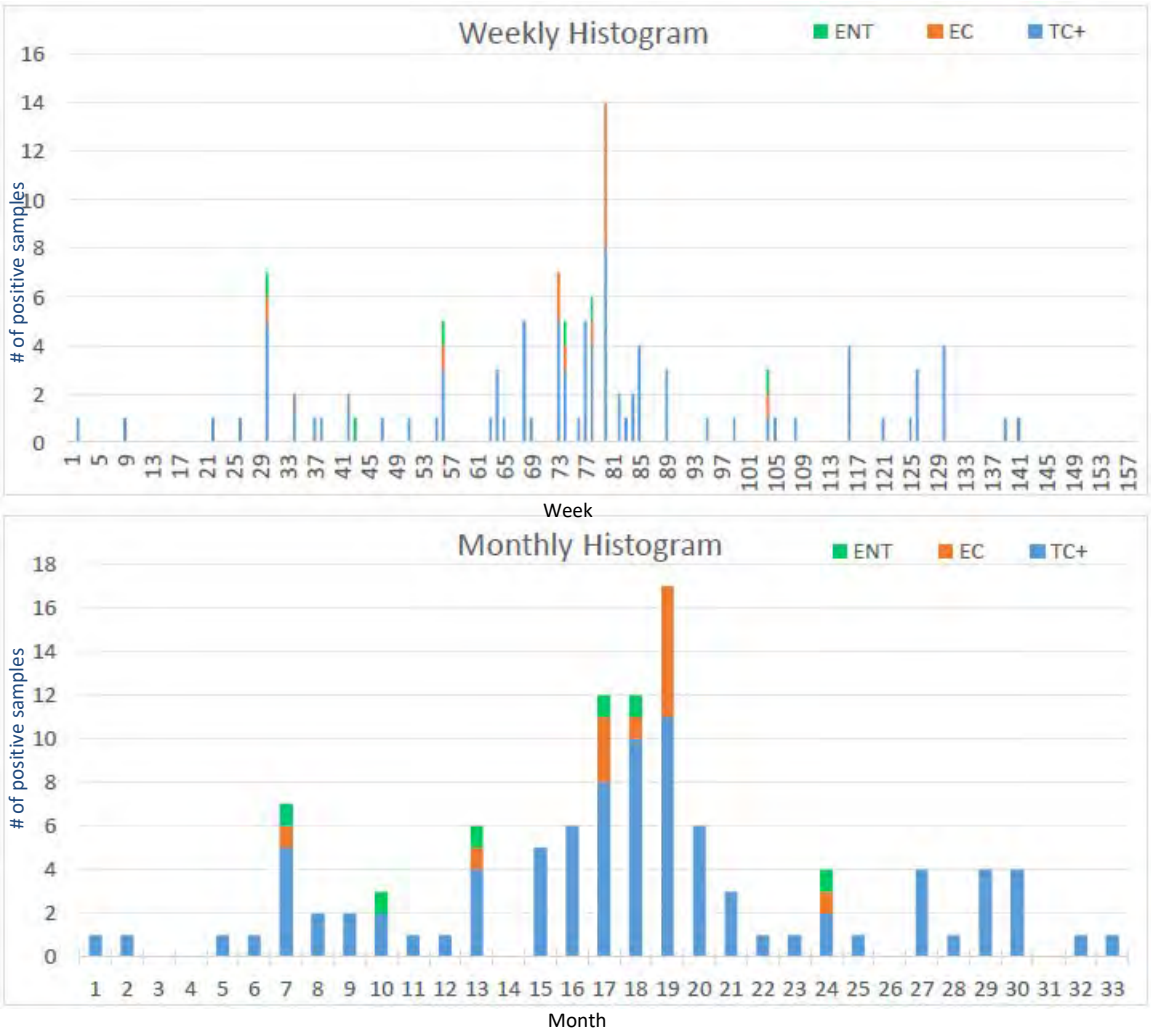

Figure 12: Weekly and monthly histograms displaying the number of positive samples from April 2015 to December 2017 for each consecutive week or month respectively. ENT indicates Enterococcus positive, EC indicates E. coli positive and TC+ indicates total coliform positive.

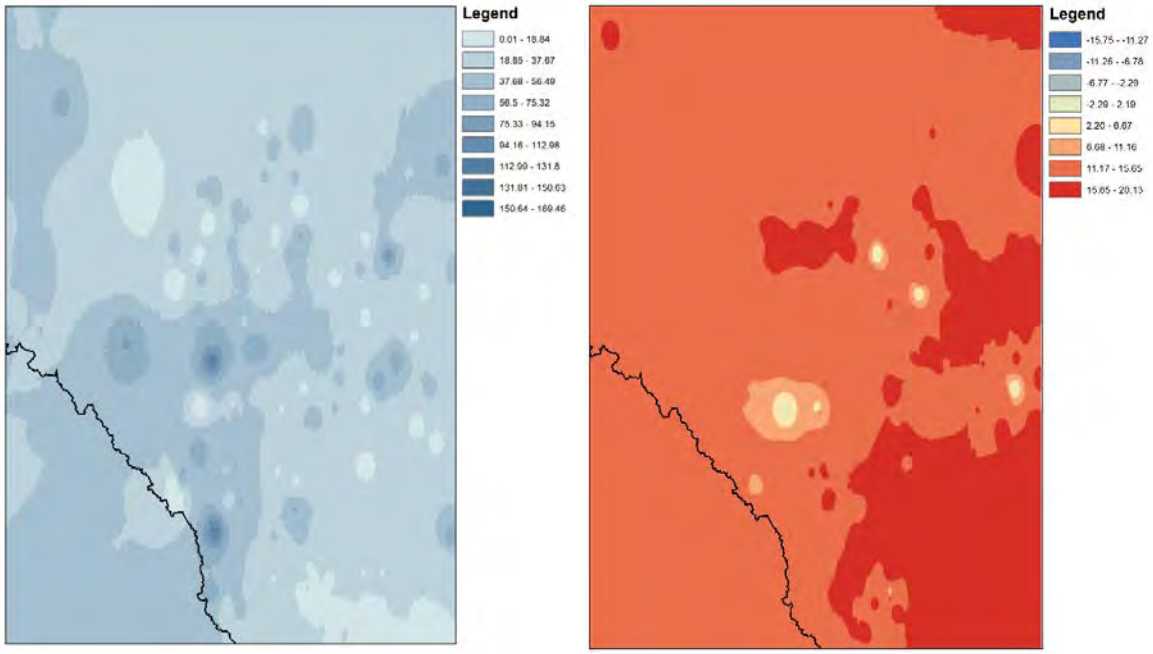

Figure 13: Precipitation (left) and temperature (right) raster graphics for June 2015

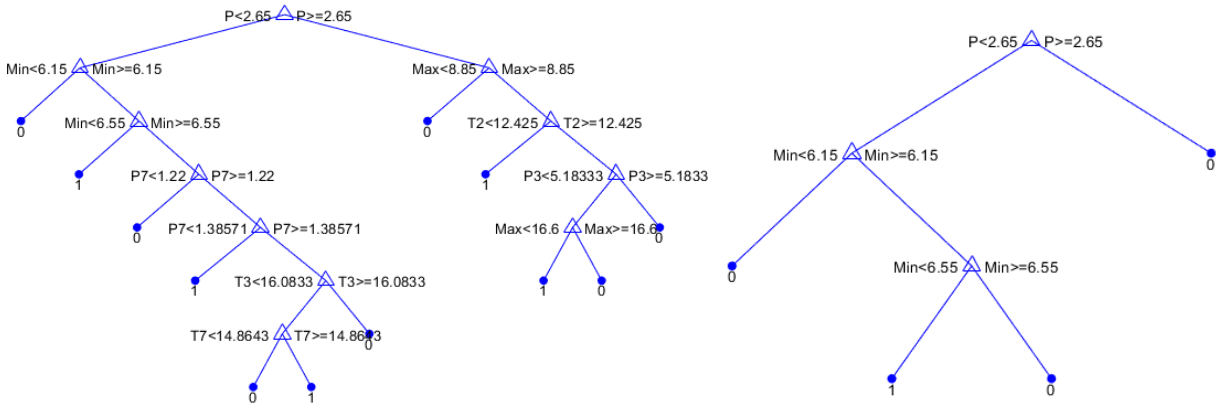

Figure 14: Medium tree (left) and coarse tree (right) classification models

Key Learnings

Please provide a narrative that discusses the key learnings from the project.

- Describe the project learnings and importance of those learnings within the project scope. Use milestones as headings, if appropriate.
- Discuss the broader impacts of the learnings to the industry and beyond; this may include changes to regulations, policies, and approval and permitting processes

RESPOND BELOW

The key learnings from each of the sub projects are reported.

STEC study: The purpose of the STEC study was to determine the frequency of occurrence and spatiotemporal patterns of STEC in water well samples across Alberta. From this project we learned that STEC were found in 8% of *E. coli* positive drinking water samples and 0.2% of all drinking water samples from southern Alberta between 2004-2016. STEC positive drinking water samples were frequently found to contain the 'big-six' serotypes causing human clinical infections, and for which some isolates displayed antimicrobial resistance. STEC occurrence in groundwater drinking wells varied by year and season, but importantly, displayed temporal patterns consistent with that observed for clinical cases, suggesting that drinking groundwater may be an extremely important but under-rated risk factor for STEC-related disease. *E. coli* isolated from drinking water wells also showed significant temporal patterns. *E. coli* isolated from drinking water wells also showed significant temporal patterns. In addition, STEC and *E. coli* with AMR isolated from groundwater wells also displays spatiotemporal variation suggesting that residents in some areas of southern Alberta are at greater risk of contamination of their drinking water with STEC or AMR and during certain times of the year.

AMR study: Understanding the geospatial patterns of AMR and MCR *E. coli* within Alberta's rural well water may provide well owners and policymakers with information to assess risks of AMR *E. coli* contaminant within the province. This information can be used to identify locations where interventions are needed, and guide policy decisions on water testing and treatment requirements in Canada. Understanding the spatial and temporal distributions of AMR *E. coli* may provide insight into clinical and surveillance data in humans and animals, and be used to interpret potential outbreak data in the future. These spatial analyses are performed using locations provided by the well owners when they submit their samples. In this study, only 60% included locational information. Requiring locational information inclusion with well samples for testing would increase the accuracy and decrease bias in these analyses.

Recruitment study: There was generally a low uptake of participation in the study amongst clients of the veterinary clinics tasked with the initial recruitment drive for this study. When recruitment strategies were opened up to include acreage owners, participation increased, particularly among well owners already actively invested in informing themselves about best practices for water well management (i.e. those attending workshops). The Water Well Workshops were an excellent partner with wide reaching access

across many counties in Alberta. The attendees were more aware and responsive to well water quality guidelines than participants recruited via other methods.

We learned that there was no difference in the number of premises that tested positive for total coliforms or *E. coli* when the different types of premises were considered (feedlot, cow/calf, broiler, acreage). There is a low uptake of free water testing in Alberta among questionnaire respondents. Just 36% of respondents indicated that they regularly submitted samples for bacterial water testing; however, just 25% actually reported testing their water at least annually. Just 20% of respondents reported testing their water for chemicals annually. Poultry producers were more likely to test their water for bacteria annually than cow/calf producers and more likely to test their water for chemicals annually compared to cow/calf producers and acreage owners. This group were less concerned about their well water becoming contaminated. Well maintenance procedures such as shock chlorination were not undertaken regularly by most respondents; 35% had never had their well shock chlorinated. Poultry producers were more likely than acreage owners to treat their water, likely related to the fact that this group tested their water more frequently. Acreage owners were more likely to express concern about well water contamination than livestock producers, however expressing concern about the potential for contamination did not mean respondents were more likely to conduct bacterial testing, chemical testing or shock chlorination. In addition, with 20/97 wells positive for contamination, there is concern about the potential risks to human health when consuming water from groundwater sources.

Virology study: The study demonstrates that the groundwater in Alberta's sentinel site has low-level contamination with enteric viruses. There was no association of the presence of enteric viruses in the well water samples and total coliforms or *E. coli* positivity in tap water samples at the same premises, further evidencing the lack of correlation between presence of enteric viruses and bacteriological indicators (Wu et al, 2011). There was no correlation between contamination of enteric viruses in groundwater and livestock operations.

Extended pathogen testing, source tracking and persistent contamination study: There is a strong relationship between the detection of *E. coli* via standard culture methods and the detection of *Bacteroides* via the GenBac3 marker and *Enterococcus* via the Entero1 marker. Both *Bacteroides* and *Enterococcus* were frequently detected in private well water samples, despite the absence of culturable *E. coli*. The presence of DNA from these bacteria in the water sample indicate that at some point either the well or the distribution system has been compromised, and that this may be with faecal bacteria. It is important to take into account that the presence of these DNA markers does not mean that there were live bacteria in the water at the time of sampling, as the methodology used did not differentiate between the presence of live and dead bacteria. Overall, the results indicate that the use of molecular markers such as *Bacteroides* or *Enterococcus* may be more useful indicators of well vulnerability to contamination than the use of *E. coli* culture alone.

This study emphasises the value of undertaking regular water testing over an extended period of time. Wells from which negative samples were at the first sampling were likely to test positive for total coliforms when further regular sampling was carried out over an extended duration. The use of extended pathogen screening also indicated that even though wells may test negative for total coliforms or *E. coli*

they may still be vulnerable to contamination, evidenced through the number of samples from which *E. coli, Bacteroides* or *Enterococcus* were detected in the absence of standard culturable faecal indicator bacteria. Where *Bacteroides* were detected the number of samples from which human faecal contamination was detected in this study was very low.

Perceptions study: The aim of this study was to evaluate well owners' perceptions of risks to their well water supply. There is a perceived low risk of well water contamination by participants. Participants consider that if contamination did occur it could have severe health affects on humans and livestock. Well water testing was viewed as a diagnostic measure protective of health that also provides peace of mind that water is safe enough to drink. The no charge water testing policy was viewed as important for increasing rates of water testing in Alberta although drop-off hours were seen as a barrier. Most participants were comfortable with quality of their well water.

Vulnerability mapping study: The aim of this study was to evaluate vulnerability factors that specifically relate to bacterial fate and transport in shallow aquifers intrinsically vulnerable to bacterial contamination. An intrinsic aquifer vulnerability map for bacterial contaminants was developed by including climatic, soil and geologic characteristics as vulnerability factors. The inclusion of climatic factors that can influence soil conditions make this vulnerability map unique. Only two factors, cold season soil moisture and growing season precipitation were found to have a significant relationship to bacterial detection. Further testing to incorporate contaminant sources would allow for better understanding of the relationship between vulnerability and bacterial detection. While the final mapping completed cannot be used to predict contamination it can be used to provide a basic understanding of the intrinsic vulnerability of shallow aquifers to bacterial contamination.

VRAT study: The aim of the VRAT was to characterise wells that may be vulnerable to contamination at the wellhead or in the surrounding lithology. This was a pilot study to test the application of the VRAT in a field setting. VRAT hardly missed identifying a site that tested positive for bacteria and viruses. However, often times, VRAT identified threats with a well, but the bacteriological and virology samples tested negative. This may be due to irregular sampling or not sampling after events such as periods of high precipitation. Although VRAT identified a threat but the water sample did not indicate any contamination, it is likely that the threats identified are still valid and contamination may be identified at another time. It is reasonable to mitigate the identified risks, especially as past research has confirmed threats including shallow wells, missing and loose well caps, cracked casing, non-grouted casing, and proximity to septic systems, livestock, and manure storage. VRAT is more effective in identifying wells vulnerable to contamination, compared to traditional practices of using total coliforms (Gonzales, 2008). The VRAT shows that well construction has an important influence on whether drinking water could potentially be contaminated. This risk assessment approach to identifying wells at risk of contamination can be used by Public Health officers to determine insufficiencies in the well and possible sources of contamination in the surrounding areas.

This pilot study was a collaboration between researchers at the University of Calgary and AHS public health inspectors. The AHS had been developing the VRAT for use when assessing approximately

2000 municipal public wells in Alberta. Piloting the VRAT within the context of this research study allowed for testing of the tool within a field environment. Access to wells on which extended pathogen testing was undertaken was invaluable, indicating that contamination may be present in the wells that is not identified using regular testing. The rigorous process of utilising the VRAT in a scientific study allowed for identification of knowledge gaps and problems with the tool. The tool has since been further developed to be more conservative and now includes factors such as angles from point sources such as manure to the well screen. The process has also become more automated, addressing the intensive time requirements that went into preparing data for utilization in the VRAT.

Faucet study: The outcome of this study was counter-intuitive. It was expected that the use of 'optimal' sampling locations, and the disinfection of faucets prior to sampling would mitigate sources of potential contamination. However, this was not the case. There was no statistically significant difference in the number of samples from which contamination was detected before and after the intervention and the frequency of contamination detected actually increased from 11/21 faucets to 15/21 faucets. It is thought that this could be because the process of disinfection increases the possibility of cleaving off of some molecular particles from colonies growing in the system. There was also the potential for cross-contamination via the use of the camera at multiple sites. More research needs to be done regarding the cause of increased positive samples after disinfection. Future studies should be aware of this possible influence that cleaning and disinfection may have.

This study was undertaken in collaboration with AHS public health inspectors. When a water sample tests positive for total coliforms the general standard approach is to request another sample of the water. The first sample is considered a false positive based on the assumption that contamination may have occurred during the sampling process if the sampling procedure was not followed correctly. The results of the faucet study indicate that this may not be the case; second samples taken following the disinfection process were just as likely to be positive. This could indicate that; a) the water is actually contaminated; b) the disinfection procedure was not successful for reasons discussed above. At this point we do not know which is the case. Further studies with a larger sample size would be useful to investigate this further.

Creation of a Tableau interface study: The Tableau interface allowed users on the research team and within AHS to explore demographic information provided in the recruitment study questionnaire in conjunction with the results of concurrent water test results. The interface allows for presentation of data in a very user-friendly format.

Rare event data study: Due to the low number of positive samples the analyses overall were inconclusive. The mapping software provides an idea of locations with increased likelihood of *E. coli* or total coliform positive samples. The multinomial logistic regression models for *E. coli* indicated that high average temperatures in the week leading up to sample collection increased the chances of total coliform growth. Similarly it was found that high average temperatures preceding sample collection increased the likelihood of *E. coli* growth and that high max temperatures on the date of collection decreased the likelihood of *E. coli* collection. This information can be used to alert well owners of increased risk of bacterial growth when higher temperatures are experienced for several days. The results of this study suggest that wells should be observed closely when several days of warm weather are experienced,

especially when followed by a colder day. This information could be used to mitigate bacterial growth by adjusting treatment and sampling protocols of well waters based on environmental monitoring.

D. OUTCOMES AND IMPACTS

Please provide a narrative outlining the project's outcomes. Please use sub-headings as appropriate.

- **Project Outcomes and Impacts:** Describe how the outcomes of the project have impacted the technology or knowledge gap identified.
- **Clean Energy Metrics:** Describe how the project outcomes impact the Clean Energy Metrics as described in the *Work Plan, Budget and Metrics* workbook. Discuss any changes or updates to these metrics and the driving forces behind the change. Include any mitigation strategies that might be needed if the changes result in negative impacts.
- **Program Specific Metrics:** Describe how the project outcomes impact the Program Metrics as described in the *Work Plan, Budget and Metrics* workbook. Discuss any changes or updates to these metrics and the driving forces behind the change. Include any mitigation strategies that might be needed if the changes result in negative impacts.
- **Project Outputs:** List of all obtained patents, published books, journal articles, conference presentations, student theses, etc., based on work conducted during the project. As appropriate, include attachments.

RESPOND BELOW

Project Outcomes and Impacts:

Participation:

- A broad transdisciplinary team was created, bringing together expertise in engineering, water quality, environmental microbiology, economics, and epidemiology. This One Health approach included team members currently working in academia, local, provincial and federal government.
- Recruiting participants to take part in the project was challenging. Even with financial incentives livestock producers were not always willing to participate, and the use of financial incentives may have biased the responses.
- Outreach through water well working groups resulted in higher participation. These participants are people who are actively considering the quality and protection of their water well.
- Broiler farmers were more likely to test their water on a regular basis due to the terms of the On-farm food safety assurance scheme set out by the Chicken Farmers of Canada

Clean Energy Metrics: Not applicable

Program Specific Metrics:

Water innovation program metric – Water Quality Protection

The results of this project are of relevance to the following polices and programs:

Project Outputs:

Publications

- Niamh Caffrey, David Hall, Jesse Invik, Edwin Cey, Sheryl Gow, Susan Cork, Katarina Pintar, Jessica Popadynetz, Caterina Valeo, Jess Nakaska, Norman Neumann & Sylvia Checkley (2020) Current practices in private water well management in Rural Central Alberta, Canadian Water Resources Journal / Revue canadienne des ressources hydriques, DOI: 10.1080/07011784.2020.1754294
- Colin Reynolds, Sylvia Checkley, Linda Chui, Simon Otto, Norman Neumann, 2020. Evaluating the risks associated with Shiga-toxin-producing Escherichia coli (STEC) in private well waters in Canada. Can J Microbiol. May;66(5):337-350. doi: 10.1139/cjm-2019-0329.
- Abraham Munene, Jocelyn Lockyer, Sylvia Checkley & David C. Hall (2019) Perceptions of drinking water quality from private wells in Alberta: A qualitative study, Canadian Water Resources Journal / Revue canadienne des ressources hydriques, 44:3, 291-306, DOI: 10.1080/07011784.2019.1601599
- Munene A, Lockyer J, Checkley S, Hall DC. Exploring Well Water Testing Behaviour Through the Health Belief Model. *Environ Health Insights*. 2020;14:1178630220910143. Published 2020 Mar 11. doi:10.1177/1178630220910143
- Munene, A., Hall, D.C. Factors influencing perceptions of private water quality in North America: a systematic review. *Syst Rev* **8,** 111 (2019). https://doi.org/10.1186/s13643-019-1013-9
- Tamara Van Staden, Edwin Cey, Cathy Ryan, Sylvia Checkley (2018) Assessing and Mapping Groundwater Vulnerability to Bacteria in Alberta, Journal of Undergraduate Research in Alberta (JURA). Volume 7. 2018-19). https://journalhosting.ucalgary.ca/index.php/jura/issue/view/5150/43

Oral Presentations

- Checkley SL, Reynolds C, Meyer KE, Chui L, Louie M, Invik J, Gow S, Neumann N. Epidemiology of Shiga toxin-producing and Antimicrobial Resistant *Escherichia coli* in southern Alberta. CRWAD, Chicago, December 2019.
- Hall D, Checkley S, Munene A, Caffrey N, Burden P, Ba L Q, Whelan M, Maloney H. The Role of One Health in Addressing Clean Water Challenges. One Health Seminar Series, UCVM, April 26, 2019.
- Pang X, Qiu Y, Caffrey N, Gao T, Lee B, Neumann N, Checkley S, Detection of Enteric Viruses in Groundwater in Alberta. Environ Tech, Calgary, April 3-5, 2018
- Reynolds C, Checkley, S., Chui, L., Scott, C., Neumann, N. Spatiotemporal Patterns of Occurrence of Shiga-toxigenic *Escherichia coli* (STEC) in Submitted Non-Municipal Drinking Water from Southern Alberta, Canadian Society for Epidemiology and Biostatistics (CSEB), Banff, Alberta, Canada, June 2017
- McCarroll K, Checkley S, Louie M. Antimicrobial resistant *Escherichia coli* and extended-spectrum beta-lactamase producing *E. coli* in Alberta's rural well water. Canadian Association

of Veterinary Epidemiology and Preventive Medicine (CAVEPM), Calgary, Alberta, Canada, June 2017.

- VanStaden, T., Cey, E., Ryan, C., Assessing and mapping groundwater vulnerability to bacteria in Alberta. GeoREX, April 2017.
- McCarroll, K. Antimicrobial resistant *Escherichia coli* in Alberta's rural well water University of Calgary Campus Alberta Student Conference on Health, September 2016
- Dhaliwal I. Evaluation of a vulnerability risk assessment tool for wells. ProvLab Research Day, July 2016.
- Van Staden, T. Assessing and Mapping Groundwater Vulnerability to Bacteria in Alberta. UCVM SURE Research Day, August 2016.
- Nakaska J, Checkley S, Cey E, Chui L, Cork S, Gow S, Hall D, Jamal I, Lee B, Louie M, Neumann N, Pang X, Popadynetz J, Ryan C, Valeo C. Assessing Water Quality, Microbial Risks and Waterborne Pathogens in Rural Alberta using a One Health Framework, UCVM Beef Conference, Calgary, AB, June 2015.

Poster Presentations

- Reynolds C, Checkley SL, Chui L, Neumann N. Assessment of Shiga toxin-producing Escherichia coli (STEC) in Private Well Waters in Western Canada with a One Health approach. International One Health Congress, Saskatoon, Canada, June 2018.
- Hall DC, Munene A, Checkley S, Wuite J, Neumann N. Factors associated with willingness to test drinking well water for *E. coli* in rural Alberta. International One Health Congress, Saskatoon, Canada, June 2018.
- Caffrey N, Hall D, Cey E, Chui L, Cork S, Fleury M, Gow S, Invik J, Jamal I, Lee B, Louie M, Nakaska J, Neumann N, Pang X, Pollari F, Popadynetz J, Ryan C, Valeo C, Van Staden T, Checkley S. Management of well water for human consumption among beef producers in central Alberta. The Summit: International Symposium on Beef Cattle Welfare; UCVM Beef Cattle Conference, Calgary, June 2018.
- Caffrey N, Neumann N, Scott, C, Banting G, Checkley S., A One Health approach to detection of contamination in private well water systems in Western Canada. 5th International One Health Congress, Saskatoon, Canada June 2018.
- Caffrey, N., Checkley, S., Hall, D., Neumann, N., Cey, E., Chui, L., Cork, S., Gow, S., Jamal, I., Lee, B., Louie, M., McCarroll, K., Pang, X., Popadynetz, J., Reynold, C., Ryan, C., Valeo, C. Rural drinking water quality in Alberta: A one-health approach, CAVEPM, Calgary, June 2017.
- Checkley, S., Hall, D., Neumann, Caffrey, N., N., Cey, E., Chui, L., Cork, S., Gow, S., Jamal, I., Lee, B., Louie, M., McCarroll, K., Pang, X., Popadynetz, J., Reynold, C., Ryan, C., Valeo, C. Environmental Health Surveillance With a One Health Approach, ICAHS, 2017.
- McCarroll, K., Checkley, S., Louie, M., Rempel, B., Antimicrobial Resistant *Escherichia coli* in Rural Well Water, One Health EcoHealth, Melbourne, Australia. 2016.
- Dhaliwal I. Evaluation of a vulnerability risk assessment tool for wells. UCVM SURE Research Day, August 2016.
- Reynolds C, Neumann N, Chui L, Checkley S, McCarroll K, Ingham L, and Yuen N. Prevalence and Spatiotemporal Patterns of Occurrence of Shiga-toxin Producing *Escherichia coli* (STEC) in Submitted Private Drinking Water Samples from Southern Alberta, Canadian Society of Microbiologists Conference, Toronto, ON, June 2016.
- Reynolds, C., Neumann, N.F., Chui, L., Checkley, S., INSIGHTS, Edmonton, Alberta, Canada, 2015.

Prevalence of Shiga-toxin producing *E. coli* in Private Drinking Water Samples Submitted to the Calgary Provincial Laboratory for Public Health, November, University of Alberta, School of Public Health.

- Trigo M, Checkley S, Cey E, Chui L, Cork S, Gow S, Hall D, Jamal I, Lee B, Louie M, Nakaska J, Neumann N, Pang X, Popadynetz J, Ryan C, Valeo C. Developing an Interdisciplinary Surveillance System Methodology for Waterborne Pathogens in Groundwater using a One Health Approach, SURE Research Day, August 2015.
- Nakaska J, Checkley S, Cey E, Chui L, Cork S, Gow S, Hall D, Jamal I, Lee B, Louie M, Neumann N, Pang X, Popadynetz J, Ryan C, Valeo C. Assessing Water Quality, Microbial Risks and Waterborne Pathogens in Rural Alberta using a One Health Framework, UCVM Beef Conference, Calgary, AB, June 2015.
- Checkley S, Hall D, Cey E, Chui L, Cork S, Gow S, Jamal I, Lee B, Louie M, Nakaska JDJ, Neumann N, Pang X, Popadynetz J, Pintar K, Ryan C, Valeo C. Assessing Water Quality, Microbial Risks and Waterborne Pathogens in Rural Alberta using a One Health Framework. ALMA Future Fare, Calgary, AB, June 2015.

Theses

- Munene, A. (2019). Investigating Perceptions of Well Water Quality in Rural Alberta (Unpublished doctoral thesis). University of Calgary, Calgary, AB. https://prism.ucalgary.ca/handle/1880/110648
- Reynolds C. (2018) The Spatiotemporal Occurrence and Recovery of Shiga toxin-producing *Escherichia coli* (STEC) in Well-sourced Drinking Water from Southern Alberta, Canada. MSc Thesis. University of Alberta, Edmonton, AB
- Meyer KE. (2017) Antimicrobial resistant *Escherichia coli* in Alberta's rural well water. MSc thesis, University of Calgary, Calgary, AB. http://dx.doi.org/10.5072/PRISM/24938
- Van Staden, T., 2017, Assessing and mapping groundwater vulnerability to bacteria in Alberta, BSc thesis, University of Calgary, Calgary, AB.
- LeBlanc A, (2019) Statistical Analysis and Modelling of rare Event Data. University of Victoria, Engineering & Computer Science Co-op. Work Term Report.

Meetings

- Alberta Innovates – Water Innovation Program meeting: Epidemiology of Shiga toxin-Producing and Antimicrobial Resistant *Escherichia coli* in southern Alberta Well Water, May 22nd 2019, Matrix Hotel, Edmonton
- Alberta Innovates – Water Innovation Program meeting: Assessing Groundwater Quality, Microbial Risks and Waterborne Pathogens in Rural Alberta using a One Health Framework, May 24th 2018, Matrix Hotel, Edmonton
- Assessing well water quality team meeting – June 13th 2018 – University of Calgary.
- Alberta Innovates – Water Innovation Program meeting: Assessing Groundwater Quality, Microbial Risks and Waterborne Pathogens in Rural Alberta using a One Health Framework, May 24th 2017, Matrix Hotel, Edmonton
- Alberta Innovates – Water Innovation Program meeting: Perceptions of water quality among rural Albertans and association with livestock. May 24th 2017, Matrix Hotel, Edmonton
- Provincial FoodNet strategic planning meetings, member providing updates, inputs and receiving feedback related to the project (Quarterly)

- National FoodNet meeting, member providing updates, input and receiving feedback related to the project (Annual)
- Water quality group meeting of all collaborators was held at the University of Calgary in June 2016
- Water quality group meeting of all collaborators was held at the University of Calgary in October 2015

Lay articles

- Feedlot Health Management Services Producer Bulletin: http://www.feedlothealth.com/wp- content/uploads/2015/08/Feedlot-Health-AMR-AMU-Summary.pdf "
- "Alberta researchers collaborate to better understand potential microbial hazards in rural drinking water". http://watercanada.net/2015/one-health/. Published online Water Canada.
- "UCVM members research water quality in rural Alberta". http://vet.ucalgary.ca/node/2239. University of Calgary Veterinary Medicine website.
- "Researchers ask: Is Alberta well water safe to drink?" https://www.ucalgary.ca/utoday/issue/2015-01-14/researchers-ask-alberta-well-water-safe- drink. Published online UToday, January 14, 2015.
- "Well water safety focus on Alberta study". Published online CBC News, February 6, 2015. http://www.cbc.ca/news/canada/calgary/well-water-safety-focus-on-alberta-study-1.2947983. This was a short radio clip played by CBC on February 6, 2015.

Draft publications and reports

Objective 1: Retrospective study of Shiga-toxin producing Escherichia coli (STEC) and antimicrobial resistance (AMR) in E. coli positive wells

- Isolation and Recovery of Shiga toxin-producing Escherichia coli (STEC) from Groundwater Wells used for Drinking (Journal of Microbiological Methods)
- Antimicrobial Resistance and Extended-Spectrum Beta-Lactamase Production Among *Escherichia coli* from rural well water in Alberta, Canada (Applied Environmental Microbiology)

Objective 2: Prospective characterization of samples across the sentinel site

- Comparison of molecular source tracking, to conventional drinking water quality indicators in groundwater in Alberta, Canada. (Intend to submit to 'Water Research')
- Frequency and quantity of human enteric viruses detected in well-water samples collected monthly from the rural area of southern Alberta and comparison with that in surface water in Alberta (Intend to submit to 'Water Research').
- An investigation into persistent contamination of water wells in rural central Alberta (Intend to submit to Water and Health)

Objective 3: Temporal and spatial patterns of STEC, AMR and other test results

- Temporal Patterns of Groundwater Well Contamination with Shiga toxinproducing Escherichia coli (STEC) (Water Research).

- A Geotemporal and Geospatial Analysis of Shiga toxin-producing Escherichia coli (STEC) from Groundwater Wells used for Drinking (Journal Water Health).
- Spatiotemporal assessment of AMR in *E. coli* isolated from untreated groundwater meant for drinking (Journal Water Health)
- Comparison of analytic methods to account for precipitation lag time associations with groundwater quality (Intend to submit to 'Journal of Contaminant Hydrology' or 'Water, Air and Soil Pollution')

Objective 4: Bacterial source tracking of *E. coli* positive wells

- (included in publication about molecular tests above)

Objective 5:

- Perceptions of beef cattle owners of the risk of drinking well water contamination and mitigation strategies related to livestock on or near their properties.

Objective 6:

- Evaluation of a vulnerability risk assessment screening tool for low flow drinking water wells: a pilot study (Intend to submit to 'Environmental Health Review')

E. BENEFITS

Please provide a narrative outline the project's benefits. Please use the subheadings of Economic, Environmental, Social and Building Innovation Capacity.

- **Economic:** Describe the project's economic benefits such as job creation, sales, improved efficiencies, development of new commercial opportunities or economic sectors, attraction of new investment, and increased exports.
- **Environmental:** Describe the project's contribution to reducing GHG emissions (direct or indirect) and improving environmental systems (atmospheric, terrestrial, aquatic, biotic, etc.) compared to the industry benchmark. Discuss benefits, impacts and/or trade-offs.
- **Social:** Describe the project's social benefits such as augmentation of recreational value, safeguarded investments, strengthened stakeholder involvement, and entrepreneurship opportunities of value for the province.
- **Building Innovation Capacity:** Describe the project's contribution to the training of highly qualified and skilled personnel (HQSP) in Alberta, their retention, and the attraction of HQSP from outside the province. Discuss the research infrastructure used or developed to complete the project.

RESPOND BELOW

Economic

This project created jobs for nine highly qualified personnel as three research assistants, two research associates, two research coordinators, and two senior technologists.

The main benefits are from job creation; however, increasing water testing could have economic benefits where impacts on health and productivity are curtailed through prevention of waterborne illness. The scale of this impact could range from hundreds of thousands to low millions of dollars depending on the scale of the potential outbreak mitigated through increased testing. Increased uptake in water testing would be a cost to the government; however, the health benefits would likely outweigh this cost.

Environmental

The knowledge gained through the evaluation of zoonotic pathogens and antimicrobial resistance patterns of bacteria present in the environment that are potentially a risk to animal and human health is a major benefit of this project.

Results indicate that indicators of potential faecal contamination can be present in wells that is not detectable via the current methods of water quality assessment. This indicates that different approaches to water quality assessment, such as qPCR testing may be an appropriate adoption in order to better monitor water quality.

Social

Direct participants in this project should have an improved understanding of the risks of drinking untreated groundwater based on the results of the testing done on their well that was reported to each well owner. They should consider the benefits of ensuring that their water source is tested on a regular basis to protect their health. This could create a carryover affect, whereby participants relay these findings to their neighbours, family and social groups. We have found that despite the efforts of the government to encourage water testing there has been very little improvement in the uptake of these services. We have also found that water well owners often do not consider water well maintenance strategies such as regular shock chlorination. Future government education programs could highlight the benefits of such regular maintenance.

This project has informed knowledge relating to the perceptions of livestock producers and acreage owners relating to risks for contamination to their water well. The role of livestock industry guidelines on best practices was evident, whereby considerations for water quality as part of the broiler industries on-farm food safety assurance scheme incorporates water quality testing. This led to broiler farmers indicating less concern about the safety of their water source than other livestock producer groups.

Building Innovation Capacity

This project allowed for the postgraduate training of two master's level students, a doctoral student, a post-doctoral fellow, and research experience for seven university students. They will be able to take these skills and apply them to jobs in the workforce, as some have already demonstrated.

Please provide a narrative outlining the next steps and recommendations for further development of the technology developed or knowledge generated from this project. If appropriate, include a description of potential follow-up projects. Please consider the following in the narrative:

- Describe the long-term plan for commercialization of the technology developed or implementation of the knowledge generated.
- Based on the project learnings, describe the related actions to be undertaken over the next two years to continue advancing the innovation.
- Describe the potential partnerships being developed to advance the development and learnings from this project.

RESPOND BELOW

F. RECOMMENDATIONS AND NEXT STEPS

STEC and AMR study recommendations

A small but significant number of private wells overall were found to be STEC positive or positive for resistant *E. coli*. This is of critical significance when we consider the number of private well owners/managers who do not sample and have their well tested regularly, and the potential severity of

human illness associated with STEC contamination of water. This information will help public health officials further understand the significance of well water contamination and the importance of guidelines and policy related to water testing. It is also critical for livestock producers, so they understand the importance of livestock and manure management related to their own water wells for animals and humans on the farm and potentially on nearby farms. Understanding temporal and spatial patterns of STEC and is critical for policy and guidelines development. This will be contribute to guidelines for routine testing and boil water orders in these areas of the province. The next step is our stakeholder meetings this fall where we will present and discuss findings and provide reports tailored to different stakeholder group.

Recruitment study recommendations: There is a need for greater outreach in informative education programs targeting rural well owners. The use of general media is more likely to reach residents not aware of testing options or engaged in actively managing their water quality rather than targeting outlets such as the Working Well programs. Mandatory bacterial water quality testing implemented as part of the 'On Farm Food Safety Assurance Program' for poultry producers made this group stand out. The potential for implementing similar mandatory programs among other producer groups could be explored.

Virology study recommendations: Processed sample pellets (in storage) should be further tested for animal enteric viruses using qPCR methods to identify possibility and levels of contamination of animal enteric viruses in groundwater in consideration of Alberta economy and One Health. Small amount of fund is required to complete this task cost-effectively.

Perceptions study recommendations: A set of recommendations was developed and has been described in journal publications but not yet incorporated into a formal policy advisory (e.g., Canadian Cattlemen's Association Standard Operating Procedures for maintaining biosecurity of drinking water wells; this is planned for fall 2020.

VRAT study recommendations: Use of a vulnerability assessment tool in combination with traditional methods for detection of well vulnerability (total coliforms and *E. coli*) is a useful way to identify wells susceptible to microbial contamination. The study highlighted the need for multiple samples over time from each site in order to adequately characterise vulnerability. Using the findings from the pilot study the VRAT is currently being revised and tested by the AHS. Future well water quality studies could look to implement the revised VRAT to assess its application in a field environment.

Faucet study recommendations: Positive drinking water results always lead to a request for a retest by public health, based on the general inference that first sample positives are often due to contamination during sampling. This small study gives us confidence that the majority of positive results are not false positives caused by sampling method as the proportion of positive hits before disinfection did not differ from the proportion of positive hits after disinfection. The faucet study has given information of importance to our public health inspectors that can be used in their daily recommendations. The study also informed interpretation of the results we from our farm study.

Rare event study recommendations: A more consistent approach to the collection of data would ensure that the number of samples taken from each well was standardised and spanned the same time period. This would allow for more complete analysis of these data. However, this is a very difficult task as the process of requires buy in and participation of well owners which is not easy to achieve. Future work using machine learning techniques available in MATLAB classification learner may be useful to explore complex methods to analyse and interpret the data created during this study

G. KNOWLEDGE DISSEMINATION

Please provide a narrative outlining how the knowledge gained from the project was or will be disseminated and the impact it may have on the industry.

RESPOND BELOW

Scientific community: The knowledge gained in this study will be disseminated to the scientific community through publications in peer reviewed journals, and conference proceedings. At the time of submitting this report there are six publications relating to this work published in peer review journals, 5 lay articles, 5 theses and there are a number of draft manuscripts being prepared for submission to journals. Aspects of this research project has been presented as either an oral talk or as a poster at 23 scientific meetings including international conferences and student research days. This has included an annual Conference of Research Workers in Animal Diseases (CRWAD) in Chicago, USA, an International One Health Congress in Saskatoon, Canada, and a One Health Eco Health conference in Melbourne, Australia.

Further knowledge dissemination:

Peer reviewed publications

Presentation at relevant conferences

Water well owners:

General: This study was promoted in an online article in 'Water Canada' in 2015, on the University of Calgary Veterinary Medicine website, the university's newsletter 'UToday' and on CBC News in 2015. The study was also promoted at two 'Working wells' workshops in 2016-2017. The study was explained to workshop participants and interested attendees were actively recruited into the study. A statement regarding the overall results and conclusions of the study will be developed and disseminated through similar sources in September/October 2020.

The results of the water testing conducted on each premises involved in the recruitment study were provided to the participant, the public health inspectors in the relevant zone, and to the veterinary clinics (where applicable). An example is provided (Appendix 4). This document outlined to each participant the outcome of all testing done on their water, including standard bacterial tests, extended qPCR tests, VRAT, and virology tests. The document provided the owner with detailed information as to the methods used to test their water, and provided a list of informative resources available in regards to groundwater. It is hoped that reviewing these test results will educate each owner as to the quality of their water source, and encourage owners to become more proactive in maintaining their water well.

Further knowledge dissemination:

- Develop statement for dissemination to the University news and media
- Develop statement for use at Working well workshops and on the Alberta Environment and Parks website information relating to water wells
- Invite media relations to stakeholder meeting to take place September/October 2020

Industry/Producer groups: The project was advertised among cattle producer groups via an article in the Feedlot Health Management Services Producer Bulletin in 2015. In 2016, the Association of Alberta Agricultural Fieldmen also advertised the recruitment study in their newsletter. The findings of the recruitment study and perceptions studyons study component of this project were presented to cattle producers at an International Symposium on Beef Cattle Welfare and the University of Calgary's Beef Cattle conference in June 2018.

Preliminary results from the perceptions study, both quantitative and qualitative, were presented at the Oldman River Watershed Community information session (April 2018) and the Red River Watershed Community information session (May 2018) as well as a University of Calgary public seminar attended by two watershed members (June 2018).

It is the intention of the research team to develop statements tailored towards the relevant producer groups (feedlot owners, cow/calf producers and broiler producers) addressing the findings of the study and how these findings are relevant to the different producer groups. A stakeholder meeting is also planned for September/October 2020. Representatives from producer groups will be invited to attend this meeting, where the project findings will be disseminated. Producer groups may reviewing the findings may consider ways they could encourage producers to test their water on a more regular basis, such as that seen in the broiler industry.

Further knowledge dissemination:

- Present research findings at upcoming conferences that are attended by producers and(or) producer representatives
- Develop recommendations targeted towards each producer and public health stakeholder group. Submit these recommendations to relevant industry representatives e.g. Canadian Cattlemen's Association, Chicken Farmers of Canada, Alberta Beef Producers, Alberta Federation of Agriculture. (as below)
- Invite producer group representatives to final stakeholder meetings for results presentation and discussion.

Government:

AHS staff: Public health inspectors were kept informed of the results of routine water sampling conducted during this project and they actively contributed to the overall project direction. In particular, inspectors were instrumental in the design and implementation of the VRAT study and the faucet study, and are co-authors on a draft publication relating to the VRAT. The information acquired in both of those studies has been actively utilised by AHS staff, who are currently revising the VRAT tool based on the findings of the pilot study. This tool allows AHS staff to assess the risk of contamination to wells based on an assessment of the vulnerability of the wellhead to point source contaminants as described in the methodology. It is a tool that will be utilised by inspectors when assessing vulnerability of approximately 2000 municipal public wells across the province, therefore the findings of the project have been instrumental in aiding the development of an important site evaluation component of their work.

Technical Advisory Council – Drinking Water (TAC-DW): There has been ongoing dialogue with members of TAC-DW for Alberta Health/Alberta Health Services

Public Health Agency of Canada (PHAC): Collaboration with PHAC is still ongoing. Information as to the prevalence of AMR and STEC in groundwater sources is pertinent to government considerations relating to risk assessment of waterborne illness in Canada. We have current ongoing projects with this key collaborator as well as Agriculture Agri-Food Canada along with Alberta Agriculture and Forestry that build on the findings from this project. Specific policy recommendations (policy papers, standard operating procedure recommendations) are pending (to be prepared by Fall 2020). The broader implications for policy are being prepared to present to stakeholders this Fall 2020 (tentatively September) at summary of findings meetings.

Further knowledge dissemination:

- Invite public health (AH, AHS, APL, PHAC) representatives to final stakeholder meetings for results presentation and discussion.
- Present and discuss the findings of the project for staff at AHS and the APL.

H. CONCLUSIONS

Please provide a narrative outlining the project conclusions.

- Ensure this summarizes the project objective, key components, results, learnings, outcomes, benefits and next steps.

RESPOND BELOW

This was a state of the art project, bringing together many professionals working across disciplines to address an important One Health issue – water quality in rural communities and impacts on human and animal health. The project provided a detailed evaluation of waterborne pathogens in rural drinking water. Advanced analysis using spatial associations, hydro-geological modelling and economic considerations were assessed to provide information that can be used to develop water policy for Albertans. GIS was used to describe patterns of STEC and AMR and pathogen detection from *E. coli* positive wells across Alberta over time. A molecular pre-screen of all water samples taken from wells in the recruitment study, and *E. coli* positive samples from regular APL submissions was undertaken and source tracking of *E. coli* positive wells was completed. A subset of the wells participating in the recruitment study were also part of a virology study which examined water samples for the presence of eight human enteric viruses. Virological assessment indicated a low prevalence of viruses in groundwater samples. A novel tool was created to sample water for viruses. Participants were content with the quality of their well water and they considered the risk of water contamination to be very low. They were pleased to have access to free water testing but considered limited drop-off hours as a barrier. A subset of wells were part of a well vulnerability risk assessment tool (VRAT) pilot project that characterized the physical characteristics of the well and related these findings to the results of microbial testing. Based on the

results of this pilot project the VRAT tool is currently being redesigned by the Alberta Health Services. A Tableau dashboard was designed to user visualization of results. Overall, this was a very successful project that will provide key recommendations for policy and decision makers concerning ground water protection.