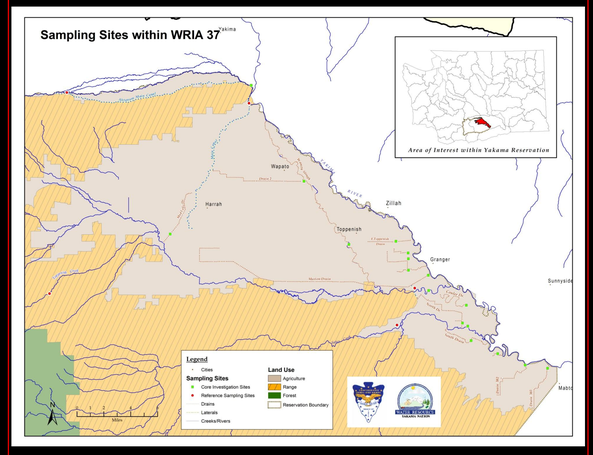
Investigation of Bacterial Contamination in Lower Yakima Valley Well Water

Aaron Etherington[1], Kazuhiro Sonoda[1], Ph. D., Nicola Beck[2], Scott Meschke[2], Ph. D.
[1]Heritage University Toppenish, WA, [2]University of Washington Seattle, WA

Introduction

❖ The Lower Yakima Valley is an agriculturally dense region in eastern Washington stretching from Union Gap to Prosser
❖ The agricultural community has been established in this area for over 150 years. This means that over a 150 years worth of additional fertilizers and dairy fecal matter have had a chance to seep into the well water
❖ Studies by the Environmental Protection Agency (EPA) have found high levels of nitrates within the ground water of the Lower Yakima Valley but little has been done to test for bacterial contamination within this region



Research Aims

❖ To assess the level of bacterial contamination with well water of the Lower Yakima Valley
❖ To help provide better insight into where the source of bacterial contamination may be coming from.

Methods

Well Water Collection

❖ Well water was collected once per sample from individual houses with the help of the EPA using sterile whirl-paks
❖ The adaptor on the well was sterilized by spraying with 70% ethanol to ensure accurate results without outside contamination.
❖ The protocol required the water to run for 5 minutes prior to collected to flush the pipes and get water straight from the well

Membrane Filtration

❖ Each sample requires the use of a sterile membrane filtration housing
❖ A millipore 47mm 0.45µm membrane filter is used to collect bacteria from a 100ml sample
❖ Each sample is run in duplicate for both selective media
❖ After each 100ml sample is filtered it is removed antiseptically and plated onto the selective media

Selective Media

❖ The selective media are made following the protocol provided by manufacture
❖ The plates used for the agar media were 60x15mm plates
❖ The selective media after being plated with the membrane filter is incubated at its designated temperature for its allotted time.
❖ Growth found on m FC plates is transferred to Nutrient Agar with MUG to test for the presence of E. Coli through fluorescence
❖ m *Enterococcus* incubates at 35°C for 48 hours
❖ m FC incubates at 41°C for 24 hours
❖ Nutrient Agar with MUG incubates at 35°C for 24 hours

PCR & Gel Electrophoresis

❖ Two human specific *Enterococcus* primers were used, HS-espF and HS-espR
❖ 23µl of master mix; a mix containing both primers, a loading dye, and H_2O; was added to each tube along with 2µl of the sample DNA
❖ The samples were then loaded into a thermocycler
❖ After completing a run in the thermocycler the samples were loaded onto a 1% Agrose gel of 1x TAE
❖ The DNA was imaged using an Ethidium Bromide stain
❖ Once all the samples and ladders were loaded into the wells of the gel it was run at 90 volts for 90 min

Acknowledgments

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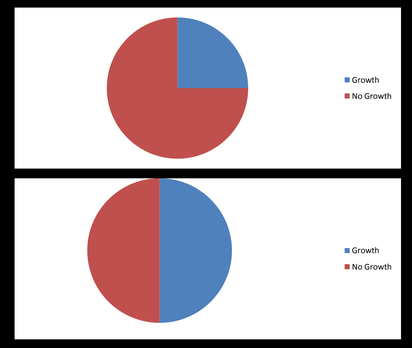
Results

❖ A total of 48 Lower Yakima Valley well water samples were collected. Of which four showed signs of possible sampling error and were thrown out when calculating the results.
❖ 11 of 44 samples contained c.f.u (<10 c.f.u./100 ml average) on m *Enterococcus* agar, while 22 of 44 samples contained c.f.u (65-90 c.f.u./100ml average) on m FC agar
(m Enterococcus on top; m FC on bottom)



❖ For the 6 (of 30) samples containing c.f.u on m *Enterococcus* no human specific *Enterococcus* genes were detected.
❖ For the 19 (of 30) samples containing c.f.u on m FC *E. coli* was present on two samples

Conclusion

❖ While the absence of human specific Enterococcus suggest the source of contamination to be from an animal source further research and analyzes would be required to more accurately assess the source
❖ Additional PCR analysis using human and bovine specific Bacteroides 16S primers will provide a greater insight into the identification of bacterial contamination sources in the Lower Yakima Valley.