

# Watershed Bio-surveillance for Identification of Pollution Sources and Potential Disease Signals

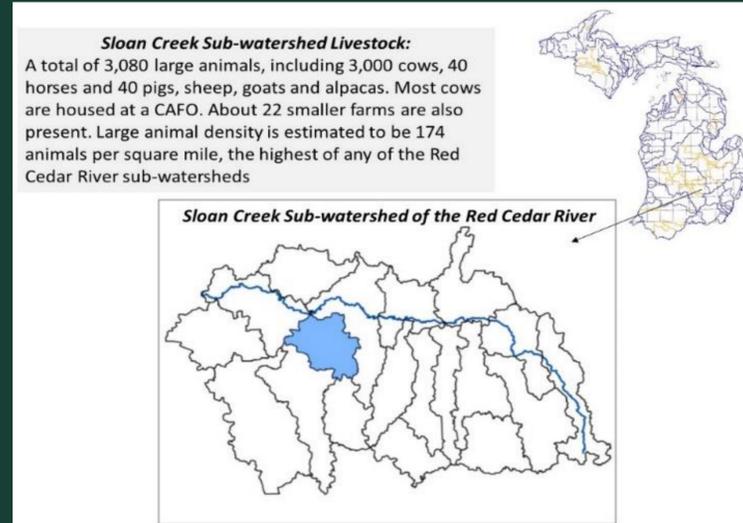
## Introduction

Early detection and prevention of livestock disease outbreaks is paramount to the animal agriculture industry. In agriculture-dominated watersheds it is impractical to test every animal for potential disease. Sampling runoff-impacted surface water from agricultural areas represents a community fecal and urine sample of the livestock population in the sub-watershed; therefore, it can serve as a screening tool for the presence of potential disease outbreaks in the corresponding livestock population. Whole genome shotgun sequencing analysis of the collected samples will provide a wide range of potential pathogens present in the sample.

Watersheds are the geographical space in which water flows, as well as a subsystem that agriculture, industrial development, population growth, climate change, and governance are of the driving forces, therefore, watersheds offer an ideal context for transdisciplinary research and seeking solutions to provide health for humans, domestic animals, wild animals, and ecosystems (Jenkins et al., 2018). Watershed bio-surveillance and management are vital in the protection of livestock health and public health.

*The objective of this research is to characterize bacterial contamination in a mid-Michigan agriculture-impacted sub-watershed. Furthermore, the goal is to provide a proposed screening method for identification of genomic sequences related to potential occurrence of cattle pathogens.*

## Site Description



Samples were collected at the mouth of Sloan Creek, right before the confluence with the Red Cedar River

## Methods

Water samples were collected in August 2015 (N=11) twice per week, with one-liter autoclaved sampling bottles. *E. coli* was measured by using Colilert -18<sup>®</sup>. Bovine-associated *bacteroides* were measured by qPCR.



Colilert -18<sup>®</sup>

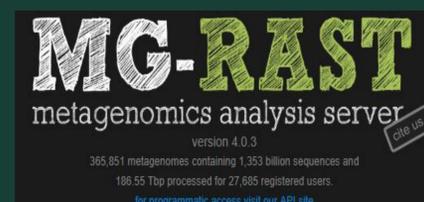


qPCR

Three samples, specifically August 15<sup>th</sup>, 18<sup>th</sup>, 19<sup>th</sup> of 2015 from Sloan Creek were processed for whole genome shotgun sequencing analysis using Illumina Miseq system. The assembled contig files for all samples were uploaded to Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) web server for analysis (Keegan et al. 2016).



Illumina Sequencing



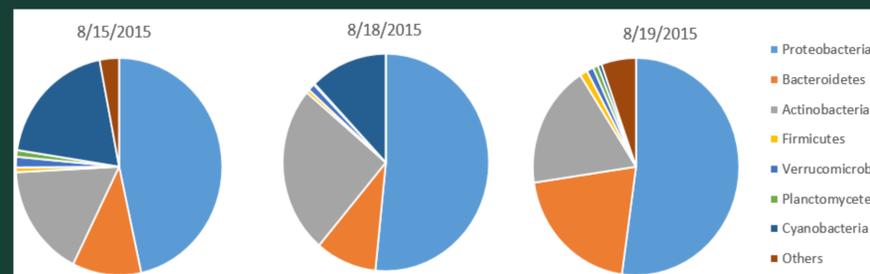
MG-RAST analysis

## Results and Discussion

*E. coli* and bovine-associated *bacteroides* were detected in the samples, as shown in the table below. In the state of Michigan, the daily maximum geometric mean is 300 *E. coli* /100 mL for total body contact recreation, and 1000 *E. coli* /100 ml for partial body contact recreation. In the majority of samples, *E.coli* concentrations exceeded the total body contact standard. The bovine fecal contamination indicator, bovine-associated *bacteroides* were detected in four out of eleven samples. High concentration of bovine-associated *bacteroides* occurred after high precipitation, indicating microbial contaminants were flushed into the stream.

Date	Discharge	Precipitation	<i>E. coli</i>	Bovine <i>bacteroides</i>
	(cms)	(mm)	(MPN/100ml)	(copies/100ml)
8/2/2015	0.018	17.3	816	12
8/3/2015	0.023	4.3	613	
8/7/2015	0.019	1.5	548	
8/8/2015	0.02	0.3	358	
8/10/2015	0.481	57.7	1046	
8/15/2015	0.054	0.8	914	1840
8/18/2015	0.034	6.1	687	3560
8/19/2015	0.045	1.8	281	
8/20/2015	0.105	10.7	411	
8/23/2015	0.065	16	462	
8/26/2015	0.062	0	770	531

MG-RAST results are characterized in the phylum distribution charts. The water samples are dominated by phylums such as *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*. Furthermore, whole genome shotgun sequencing results indicate fecal and sewer signatures (Li et al. 2015), wastewater metagenome, human gut metagenome, and rumen gut metagenome related sequences in the water samples.



The metagenome was searched for genomic sequences related to potential livestock pathogens. The potential pathogenic species were selected based on reportable cattle bacterial disease in Michigan. The results are presented as number of observed sequences (number of hits). Among others, we observed genomic sequences related to *Mycobacterium* and *Brucella* species.

Observed sequences potentially related to *mycobacterium* species are presented below. *Mycobacterium bovis* is the cause of bovine tuberculosis. *Mycobacterium avium* may be related to both human and bovine sources. *Mycobacterium bovis* is endemic in Michigan's white tailed deer and has been circulating since 1994. The strain circulating in deer has remained genotypically consistent and was detected in 2 humans in 2002 and 2004. The focal area of the endemic disease is in northern-lower Michigan. The same endemic area in northern-lower Michigan continues to exist currently in 2017 and 2018. In 2015, when the sampling described in this paper was conducted (prior to the reported spread of *Mycobacterium bovis* disease in lower western Michigan) we detected the presence of *Mycobacterium* species in Sloan Creek, in the Red Cedar River watershed, Ingham County.

	8/15/2015	8/18/2015	8/19/2015
<i>Mycobacterium abscessus</i>	82	122	49
<i>Mycobacterium avium</i>	127	194	117
<i>Mycobacterium bovis</i>	18	16	13
<i>Mycobacterium introcellulare</i>	21	14	14
<i>Mycobacterium kansasii</i>	27	27	22
<i>Mycobacterium leprae</i>	52	93	24
<i>Mycobacterium marinum</i>	46	70	42
<i>Mycobacterium parascrofulaceum</i>	27	35	30
<i>Mycobacterium tuberculosis</i>	159	207	103
<i>Mycobacterium ulcerans</i>	65	79	37

Observed sequences potentially related to *brucella* species are shown below. *Brucella* is a fastidious, aerobic, small, gram-negative coccobacillus bacterium that causes brucellosis in both animals and human. *Brucella abortus* most commonly affects cattle, *B. melitensis* is most common in goats, as well as in cattle; *B. Canine* is most common in canines; and *B. Suis* is found in swine. *B. melitensis* and *B. abortus* are the most common *Brucella* species that may affect humans. In Michigan, all *brucellosis* species are reportable. From 2007, the Michigan state received an unprecedented number of reports of *B. canis* infection, and the outbreaks continued till 2016 (Johnson et al., 2018). In 2011, six Michigan counties confirmed canine brucellosis outbreaks, including Ingham county, where the water samples were taken in 2015. In the sequenced samples, *B. abortus*, *B. Suis*, *B. melitensis*, and *B. Canine* were detected in 2015.

	8/15/2015	8/18/2015	8/19/2015
<i>Brucella abortus</i>	26	39	31
<i>Brucella canis</i>	2	12	4
<i>Brucella ceti</i>	18	16	4
<i>Brucella melitensis</i>	32	36	16
<i>Brucella microti</i>	2	4	3
<i>Brucella neotomae</i>	1	1	0
<i>Brucella ovis</i>	2	4	4
<i>Brucella pinnipedialis</i>	10	15	7
<i>Brucella suis</i>	28	35	16

## Conclusions

In this paper we report the presence of sequences potentially related to livestock associated bacteria in water samples collected from an agriculture-impacted sub-watershed in Michigan. Whole genome shotgun sequencing analysis provides data sequences of a wide range of microorganisms in the sample. The resulting information may be used as a tool for identification of potential endemic disease signals. When signals of disease of interest are observed, further testing of manure and individual animals using quantification methods, such as qPCR, is required.

## References

- Jenkins, A., Capon, A., Negin, J., Marais, B., Sorrell, T., Parkes, M., Horwitz, P., 2018. Watersheds in planetary health research and action. *Lancet Planet. Health* 2, e510–e511.
- Keegan, Kevin P., Elizabeth M. Glass, and Folker Meyer (2016) "MG-RAST, a metagenomics service for analysis of microbial community structure and function." *Microbial Environmental Genomics (MEG)*. Humana Press, New York, NY, 207–233
- Li, X., Harwood, V.J., Nayak, B., Staley, C., Sadowsky, M.J., Weidhaas, J., 2015. A Novel Microbial Source Tracking Microarray for Pathogen Detection and Fecal Source Identification in Environmental Systems. *Environ. Sci. Technol.* 49, 7319–7329.
- Johnson, C.A., Carter, T.D., Dunn, J.R., Baer, S.R., Schalow, M.M., Bellay, Y.M., Guerra, M.A., Frank, N.A., 2018. Investigation and characterization of *Brucella canis* infections in pet-quality dogs and associated human exposures during a 2007–2016 outbreak in Michigan. *J. Am. Vet. Med. Assoc.* 253, 322–336.