Exploring the distribution of waterborne pathogens *Giardia* and *Cryptosporidium* along a land-use gradient in Oregon in the context of One Health.

Mariangel Aleman Torres

Advised by Dr. Brianna Beechler, PhD Student Kathleen S. Woodley, and Veterinary Student Daniel Capasso

Major: Biology, Pre-Veterinary Medicine

Carlson College of Veterinary Medicine, Department of Biomedical Sciences

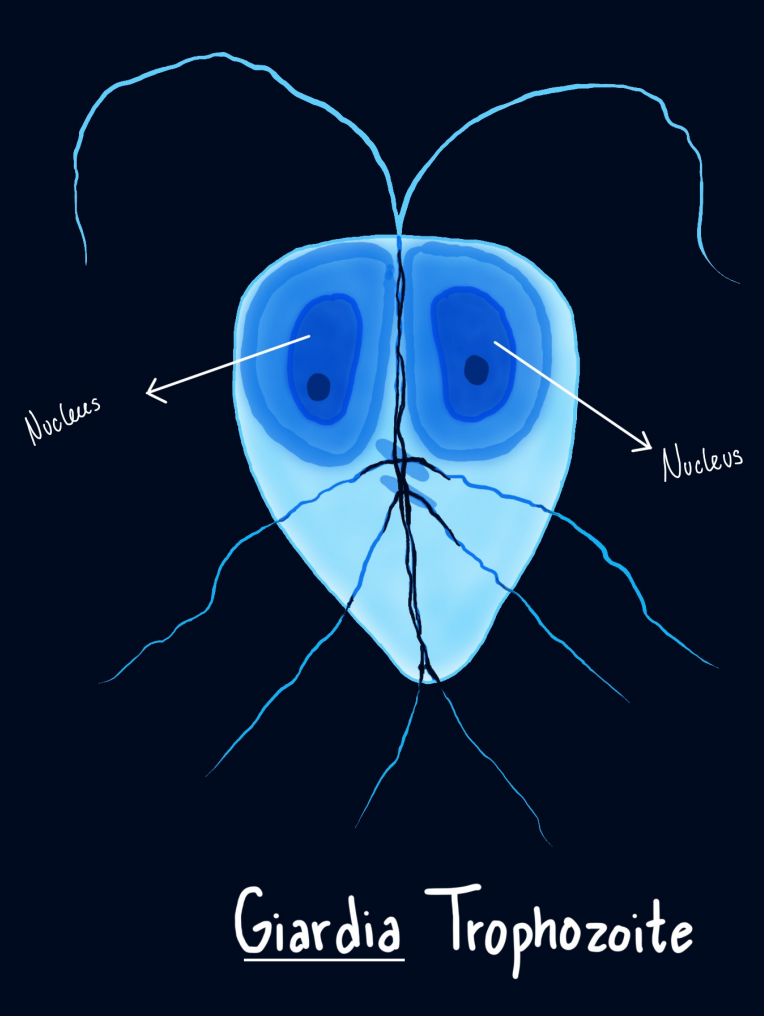
Oregon State University

Introduction to *Giardia* and *Cryptosporidium*

What is the purpose of this project?

Synopsis of *Giardia* and *Cryptosporidium*

- ***Giardia* and *Crypto* are protozoan parasites and zoonotic waterborne pathogens.**
  - Found worldwide but commonly present in ambient surface water.
  - Distributed in all mammalian, avian, and reptilian species.
  - Livestock animals are of special interest as they can have a significant impact on water supply catchments or watersheds.
  - Variety of *Giardia* and *Crypto* species but only a few have been identified to infect humans.
- **Resilient pathogens that can bypass water treatment and kill millions of people and animals annually.**
  - ~200 million people are infected with giardiasis disease every year.
  - ~748,000 people are infected with cryptosporidiosis disease every year.
- ***Giardia* cysts and *Crypto* oocysts spread through direct or indirect contact of feces to oral routes.**
  - This can occur through person-to-person contact, zoonotic, and the consumption of contaminated water and food.
  - Poor sanitation and poverty can increase the risk of human giardiasis and cryptosporidiosis diarrheal disease outbreaks in both the developing and developed world.



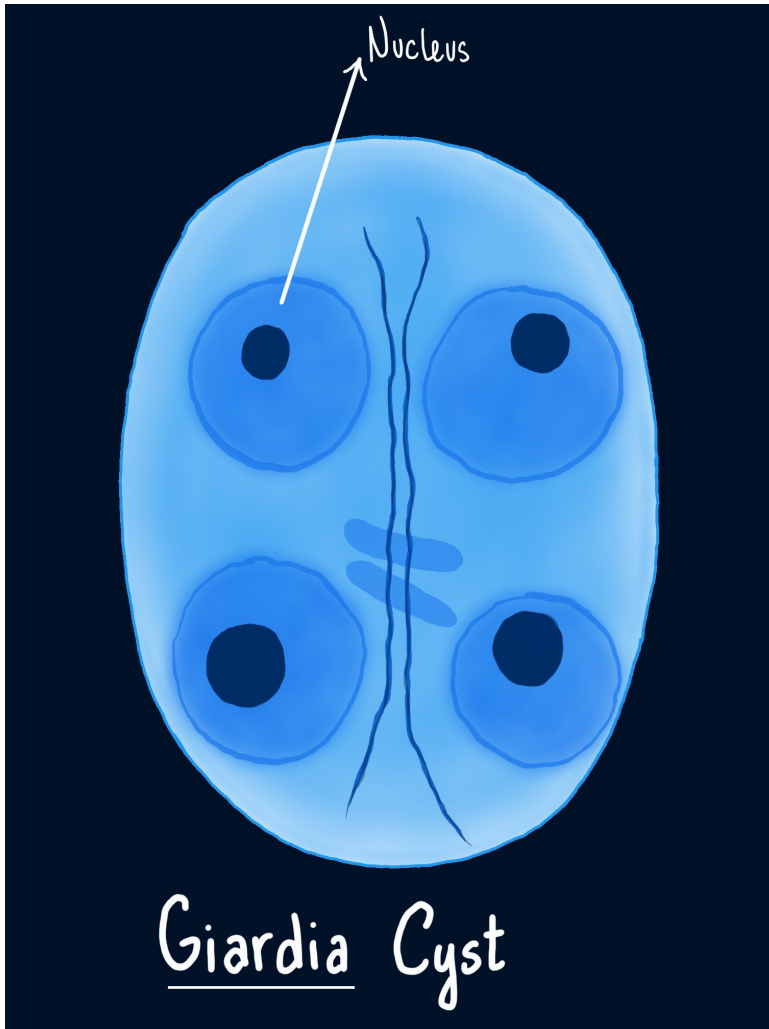
Giardia Cyst Diversity

- **Human infective species:** *Giardia duodenalis*, *Giardia lamblia*, or *Giardia intestinalis*.

- **Diplomonads:** Flagellated protozoa with double cells and 2 nuclei.
  - 4 copies of the same gene.
  - Intragenomic heterozygosity

- Large diversity within *Giardia* cysts.
  - **Assemblages:** A and B are zoonotic.
  - C-H are host specific.
  - Subassemblages (e.g., AI, AII, AIII, etc.).
  - Animals and humans can share subassemblages, a potential risk for zoonotic transmission.

- Trophozoites transition into cysts in the intestinal tract of the host and are excreted into the environment.

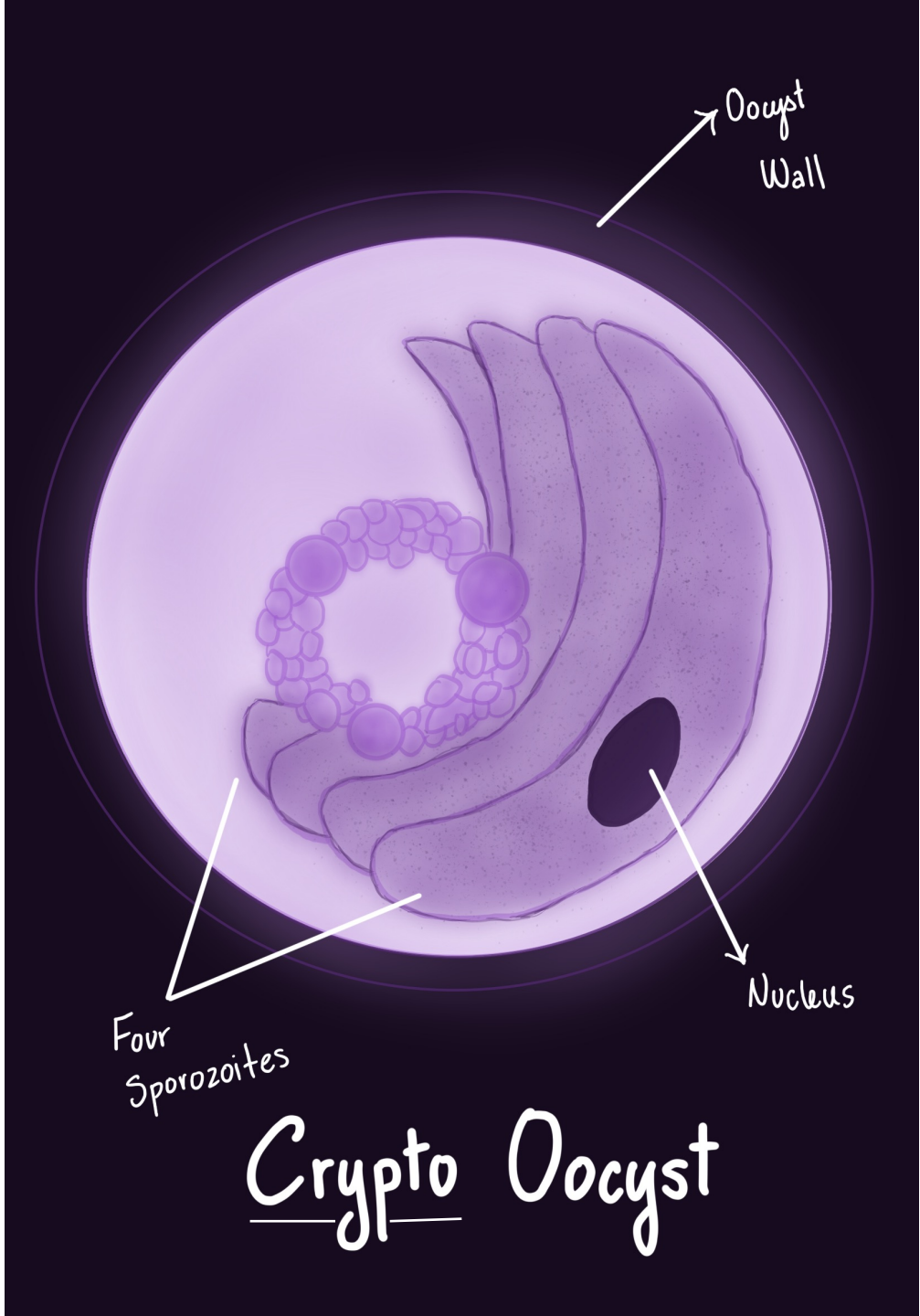


Cryptosporidium Oocyst Diversity

- **Most common human infective species:** *Cryptosporidium parvum* and *Cryptosporidium hominis*
  - **Less common:** *C. meleagridis*, *C. felis*, and *C. canis,* among others.
  - Infective species vary based on geographic location and host developmental stage.

- Variations in animals' infective species, most are host specific.
  - Location and developmental stages.
  - Genotype and subtype levels.
  - **Subtype families:** For *C. parvum*, IIa, IIb, IIc, etc., and for *C. hominis* Ia, Ib, Ic, etc.
  - Animals and humans can share subtypes, a potential risk for zoonotic transmission.

- Four sporozoites are contained within the oocyst.
  - Sporozoites are hatched at the intestinal level.



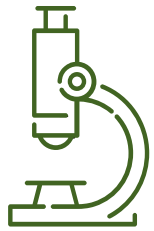
Hypothesis and Objective

1 Consider current global challenges.

2 Apply the **One Health concept** to our research study for a multidisciplinary approach.

3 Explore the distribution of *Giardia* and *Crypto* to see whether their presence and species abundance changes over a land-use gradient from **low** to **high disturbance**.

- Expect to see higher strain diversity at low disturbance sites.
- Will determine what populations of people and animals are at risk in Oregon.



Methods

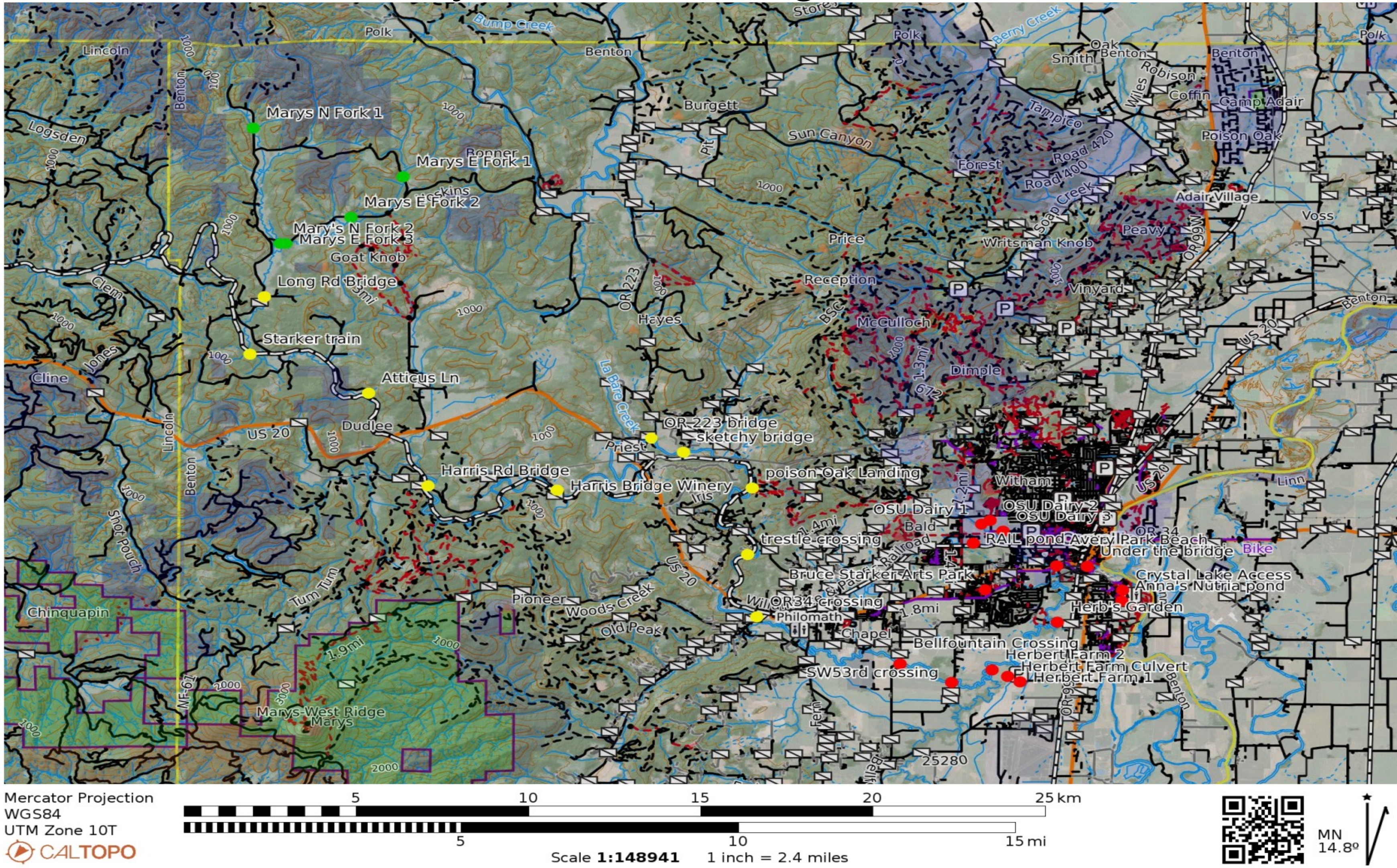
How did we approach our research study?

Sampling Sites in Oregon

Mary’s River in the Willamette Valley, Western Oregon: **24 sites**

Markers

- Starker train
- sketchy bridge
- poison Oak Landing
- OR 223 bridge
- OR34 crossing
- SW53rd crossing
- trestle crossing
- Under the bridge
- Atticus Ln
- Avery Park Beach
- Bellfountain Crossing
- Bruce Starker Arts Park
- Harris Bridge Winery
- Harris Rd Bridge
- Herb's Garden
- Herbert Farm 1
- Herbert Farm 2
- Herbert Farm Culvert
- Long Rd Bridge
- Mary's N Fork 2
- Marys E Fork 2
- Marys E Fork 3
- Marys N Fork 1
- Marys E Fork 1

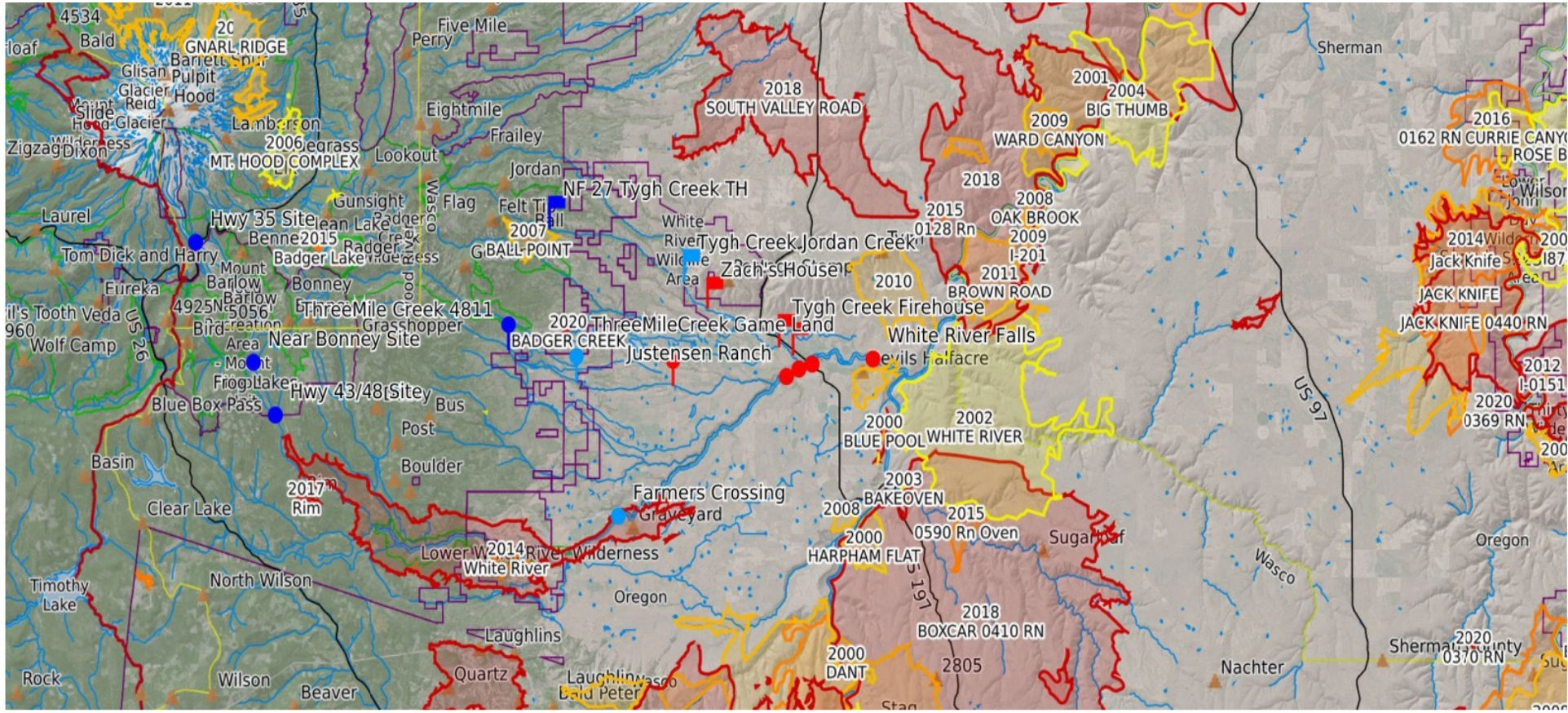


Sampling Sites in Oregon

Tygh Valley along the White River, Eastern Oregon: **19 sites**

Markers

- Elizabeths House Tygh Creek
- Farmers Crossing
- Hwy 197 Site
- Hwy 35 Site
- Hwy 43/48 Site
- Justensen Ranch
- Kate & Dan House
- Near Bonney Site
- NF 27 Tygh Creek TH
- Three Mile Creek Wamic Delayne's House
- ThreeMile Creek 4811
- ThreeMileCreek Game Land
- Tygh Creek Firehouse
- Tygh Creek Jordan Creek
- White River Falls
- Zach's House



*Three extra sites were added:

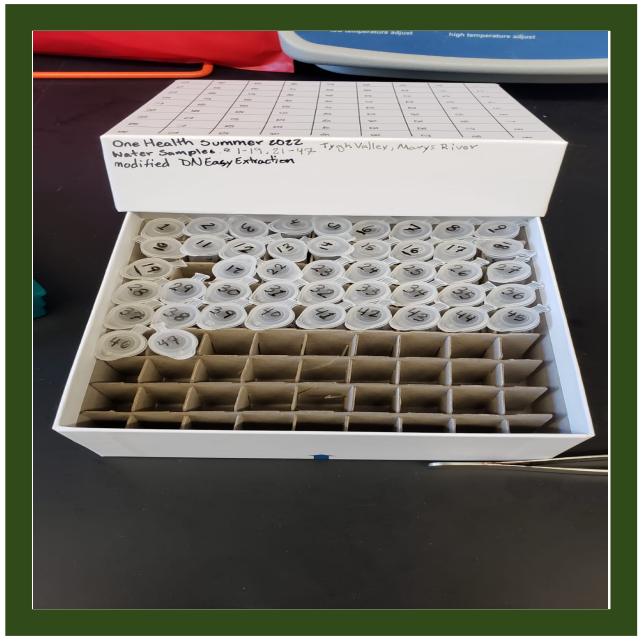
- Keeps Mill
- Near Bonney Site
- Three Mile Creek Ditch

Sample Collection

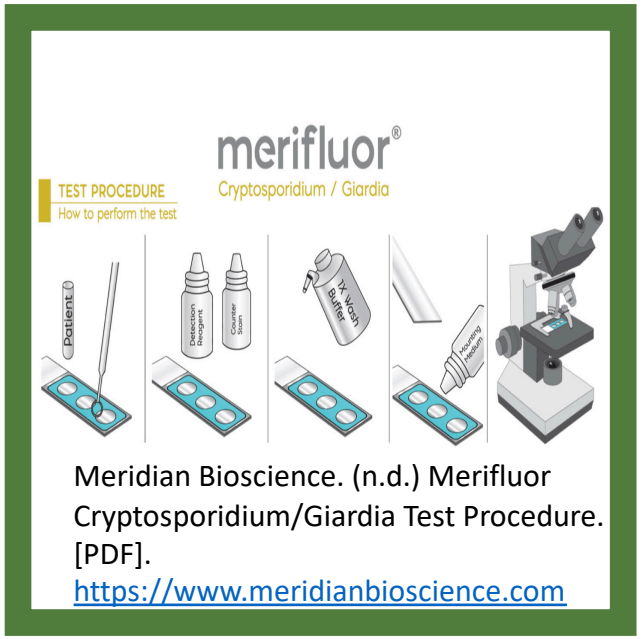
- Collected 1L of water at each site.
- Samples were kept cool and immediately transported to lab for filtering.

Laboratory Techniques

- Filtered with 0.45 µm cellulose nitrate filters.



- Aliquoted samples for Merifluor immunoassay.
- Extracted DNA for MiSeq sequencing.



Meridian Bioscience. (n.d.) Merifluor Cryptosporidium/Giardia Test Procedure. [PDF]. https://www.meridianbioscience.com

- Used Merifluor immunoassay to determine the presence/absence of Giardia and Crypto at the sampling sites.

Currently Working On:

1. PCR (Polymerase Chain Reaction)

- Genotype *Giardia* cysts from eDNA.
- Create new primers for multilocus genotyping.

2. Multilocus Genotyping

Sequence using MiSeq:

- 18S rRNA gene
- β-giardin (bg) gene
- Triosephosphate isomerase (Tpi) gene
- Glutamate dehydrogenase (Gdh) gene

3. Determine *Giardia* Species and Strain Abundance

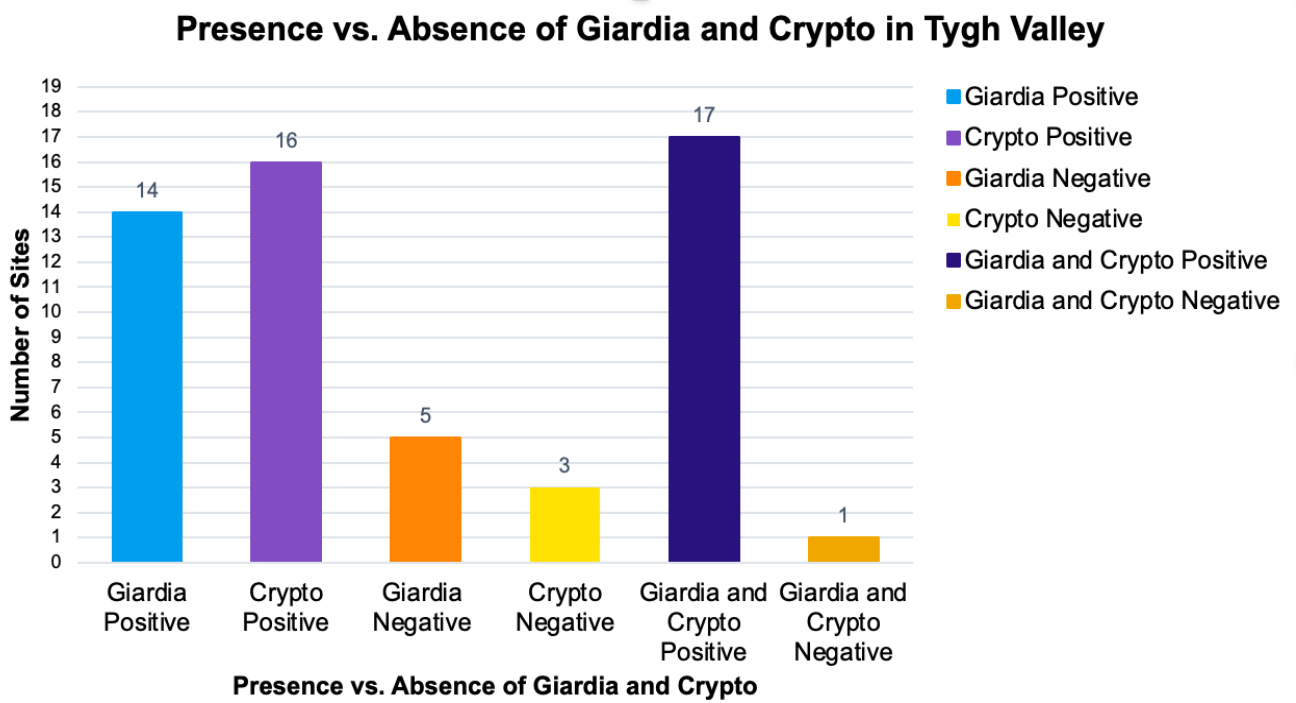
- Use BLAST and *Giardia* database.
- Generalized linear model.

Results

Current and pending results.

Tygh Valley Merifluor Immunoassay Results

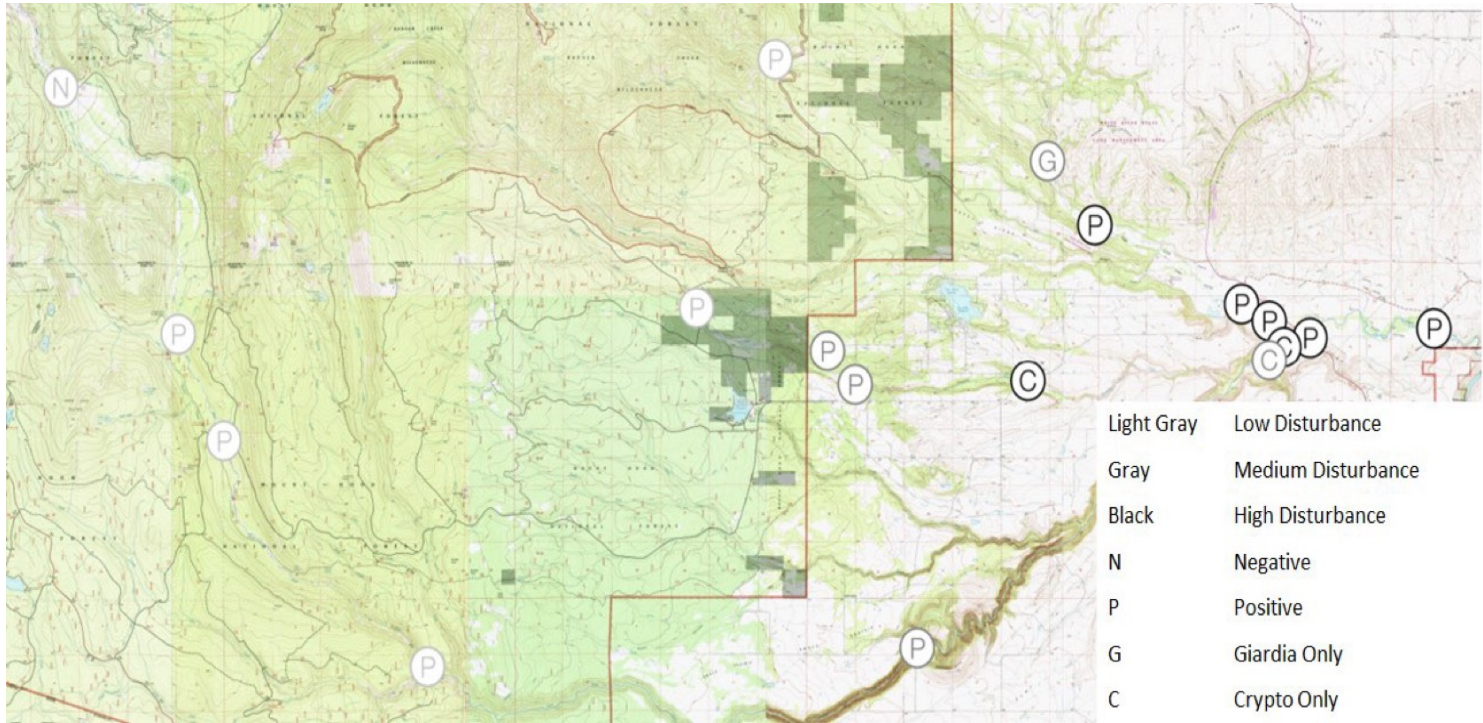
	Giardia and Crypto Positive	Giardia Negative	Crypto Negative	Giardia Positive Only	Crypto Positive Only	Giardia and Crypto Negative
Number of Sites	17	5	3	1	3	1



ANOVA Test Results:

- No significance for the presence of *Giardia* and *Crypto*.
- *Giardia* p-value: 0.684
- *Crypto* p-value: 0.507

*Procedure error occurred when doing Mary's River samples, 18S rRNA gene will be used for PCR.



Pending Results

- Determine species strain diversity for *Giardia* and relative abundance.
- Our current focal point is *Giardia* abundance.
- *Crypto* abundance will be determined in the near future.

Discussion

Why does this matter?

Long-term Goals of the Project

- Effects of anthropogenic activities on *Giardia* and *Crypto* diversity and abundance.
- Advocate and apply One Health concepts.

Limitations Experienced

- Procedural errors.
- Merifluor immunoassay.
- Not enough sample.

Conclusion and Implications

Current findings help determine which sites are a potential risk of giardiasis and cryptosporidiosis in Tygh Valley.

What are the future directions of the study and how will the findings be helpful towards the community?

- Future results will demonstrate how human disturbance affects *Giardia* and *Crypto* diversity.
- Determine what populations of animals and people are at risk in Oregon.
- Understand the abundance of assemblages of *Giardia*.
- Routine testing to predict and prevent the risk of outbreaks and disease in the community.

THANK YOU

- Dr. Brianna Beechler
- Dr. Anna Jolles
- Dr. Rhea Hanselmann
- Dr. Justin Sanders
- Dr. Michelle Steinauer
- PhD Student Kathleen S. Woodley
- A special thank you to OSU undergraduate and veterinary medicine students:
  - Gabriela Reynoso, Bryce Gabbard, Holden W. Ard, and Daniel Capasso.
- And Western University of Health Sciences veterinary medicine and medical students:
  - Alexis Ybarra, Michelle McCorkell, and Nathalie Villegas.

References

- Alderisio, K.A., Villegas, L.F., Ware, M.W., McDonald, L.A., Xiao, L., & Villegas, E.N. (2017). Differences in staining intensities affect reported occurrences and concentrations of *Giardia spp*. in surface drinking water sources. *Journal of Applied Microbiology*. 123: 1607-1613. doi: 10.1111/jam.13585
- Cacciò, S.M., Beck, R., Lalle, M., Marinculic, A., & Pozio, E. (2008). Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. *International Journal for Parasitology*. 38(2008): 1523-1531. doi: 10.1016/j.ijpara.2008.04.008
- Capewell, P., Krumrie, S., Katzer, F., Alexander, C.L., & Weir, W. (2021). Molecular epidemiology of *Giardia* infections in genomic era. *Trends in Parasitology*. 37(2): 142-153. doi: https://doi.org/10.1016/j.pt.2020.09.013
- Centers for Disease Control and Prevention. (2019, July 1). *Parasites – Cryptosporidium (also known as "Crypto")*. https://www.cdc.gov/parasites/crypto/
- Efstratiou, A., Ongerth, J., & Karanis, P. (2017). Evolution of monitoring for *Giardia* and *Cryptosporidium* in water. *Water Research*. 123(2017): 96-112. doi: http://dx.doi.org/10.1016/j.watres.2017.06.042
- Erlandsen, S.L., Sherlock, L.A., & Bemrick, W.J. (1990). The detection of *Giardia muris* and *Giardia lamblia* cysts by immunofluorescence in animal tissues and fecal samples subjected to cycles of freezing and thawing. *The Journal of Parasitology*. 76(2): 267-271. https://www.jstor.org/stable/3283031
- Gerace, E., Lo Presti, V.D.M., & Biondo C. (2019). *Cryptosporidium* infection: Epidemiology, pathogenesis, and differential diagnosis. *European Journal of Microbiology & Immunology*. 9(4): 119-123. doi: 10.1556/1886.2019.00019

References

- United States Environmental Protection Agency. (2000, September). *Giardia: Drinking water fact sheet*. https://www.epa.gov/sites/default/files/2015-10/documents/giardia-factsheet.pdf
- Xiao, L. (2009). Molecular epidemiology of cryptosporidiosis: An update. *Experimental Parasitology*. 124(2010): 80-89. doi: https://doi.org/10.1016/j.exppara.2009.03.018
- Xiao, L., Morgan, U.M., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R.C.A., Fayer, R., & Lal, A.A. (1999). Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. *Applied and Environmental Microbiology*. 65(8): 3386-3391. doi: 10.1128/aem.65.8.3386-3391.1999.
- Template design and template design images were generated by Oregon State University.
- Other images were taken during the research project by team members and me.
- Giardia and crypto illustrations/diagrams were created by me using a digital sketchbook.
- All maps were developed using CALTOPO.