



Detection of Pathogenic *Leptospira* DNA in Mānoa Stream

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Introduction

Leptospira species

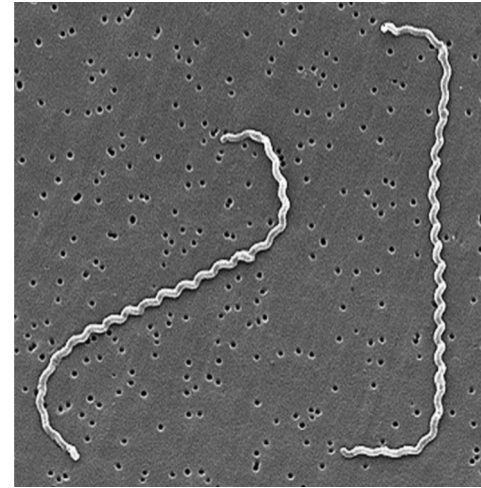
Spirochete, 0.1 μm by 6 μm - 20 μm

13 species of *Leptospira*, contains pathogenic & non-pathogenic species

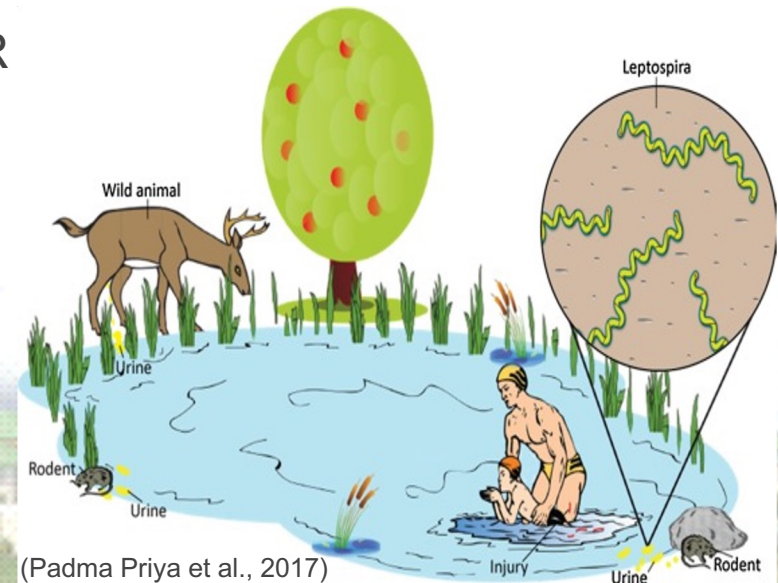
Pathogenic *Leptospira* species express the *lipL32* gene, which will be detected during qPCR

Survives best in tropical, wet environment, 28-30°C

Primary animal reservoirs of *Leptospira* in Hawaii: rodents and mongooses



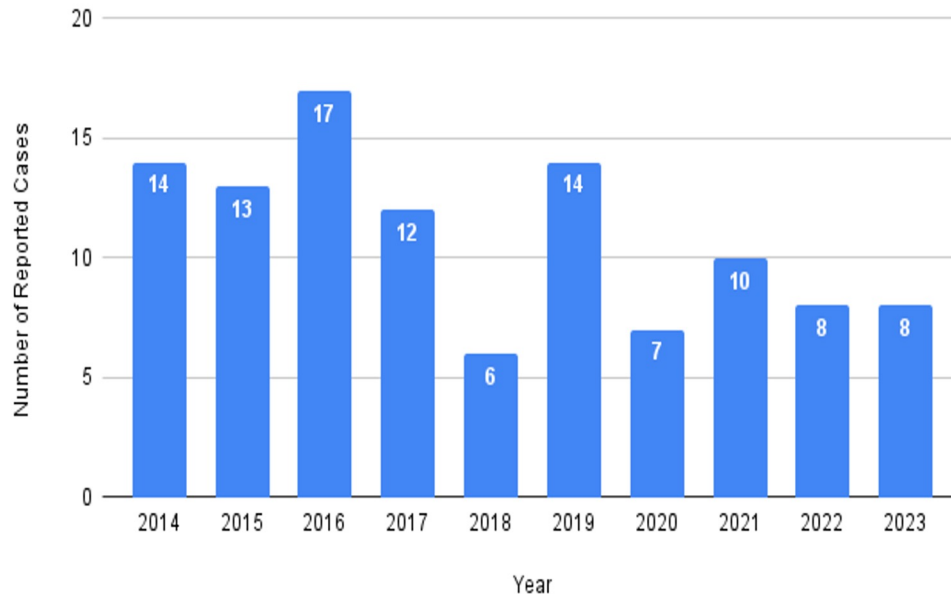
(Weyant, R. S., S. L. Bragg, and A. F. Kaufman.)



(Padma Priya et al., 2017)

Prevalence in Hawai'i

Reported Leptospirosis Cases in Residents and Non-Residents in the State of Hawaii from 2014-2023



(Data from HDOH, Disease Outbreak Control Division)

2004 UH Mānoa Flood

At least two or more UH employees acquired leptospirosis after Manoa stream flooded the UH campus

Both individuals went in a flooded lab with abrasions on their feet and tested positive for leptospirosis.



(University of Hawai'i at Manoa)

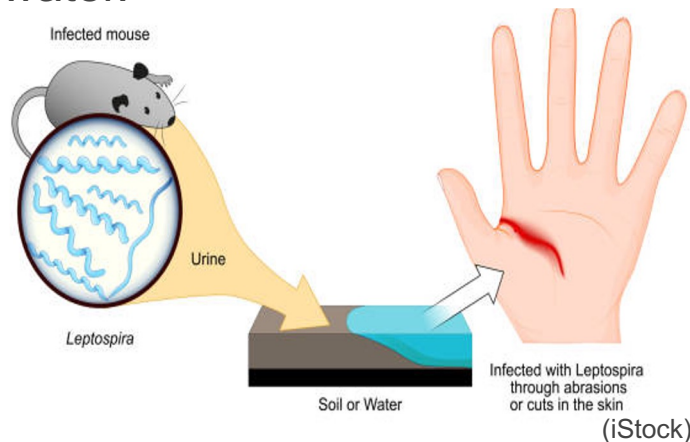
Leptospirosis

An infection caused by pathogenic *Leptospira* spp..

Commonly spread through recreational/occupational exposures.

Infection occurs via contact with urine from infected animals.

Through cuts in the skin or mucosal membrane, or prolonged immersion in water.



Symptoms of Leptospirosis



High fever.



Red eyes.



Abdominal pain.



Headache and muscle aches.



Chills.



Rash



Nausea, vomiting and diarrhea.



Yellow skin or eyes (jaundice).



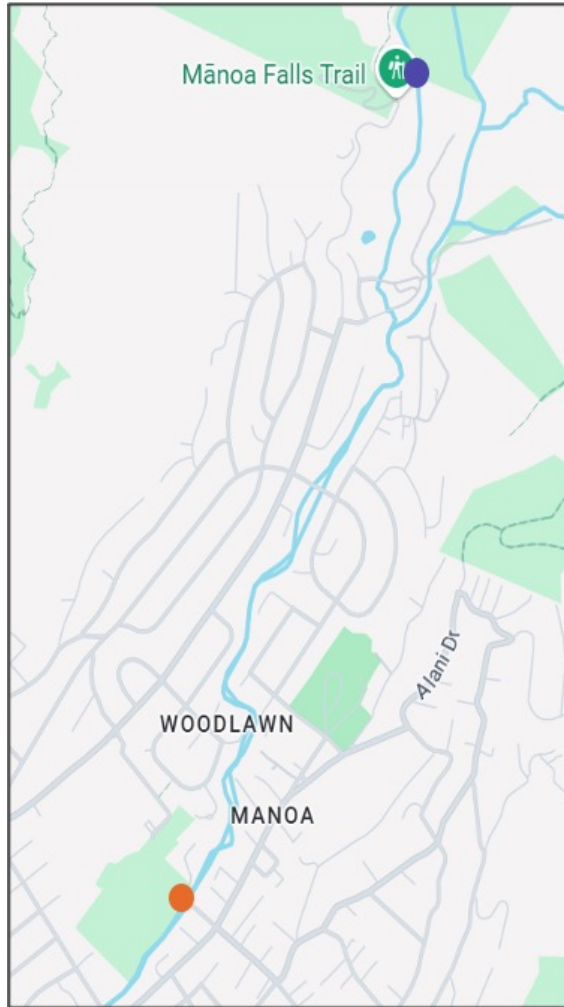
Go to the nearest emergency room if you are coughing up blood or have blood in your poop or pee.

Research Question

Is pathogenic *Leptospira* DNA present in the
Mānoa Stream?



Methods



01

Collect Water Samples

Two-liter water samples collected from two sites along the Manoa Stream.

- Two samples collected from site 1
- One sample from site 2



Site 1 ●



Site 2 ●

02

Filter Water Samples

Filter water sample through sterile gauze, then through 0.22 μ m filter



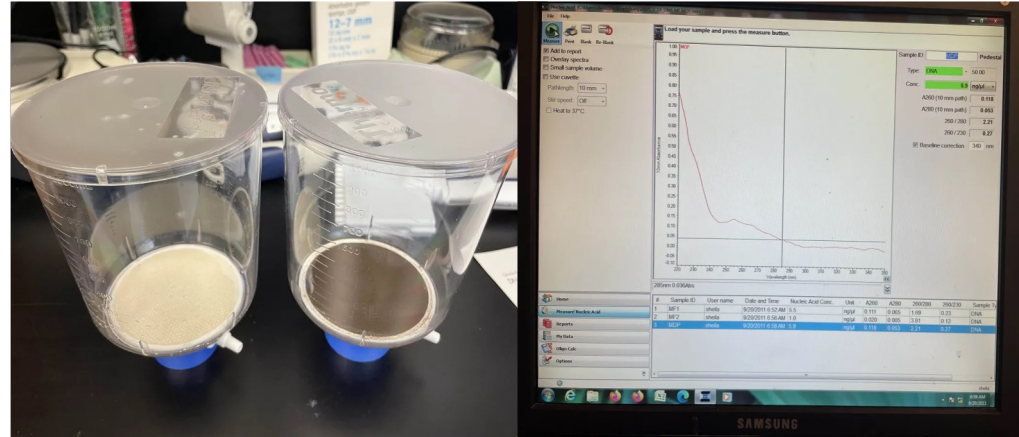
Methods

03 DNA Extraction

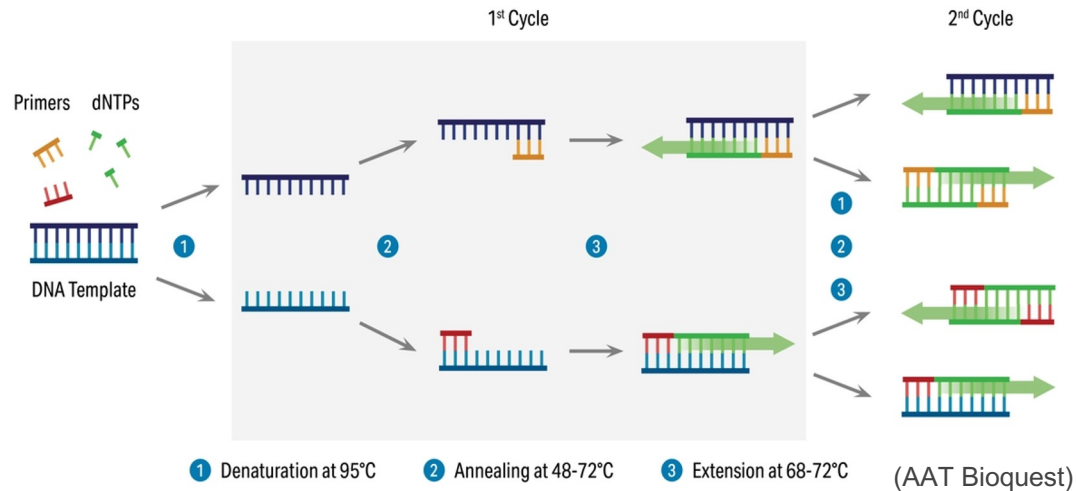
DNA is extracted from the filter membrane using the PowerWater DNA extraction kit

04 Analyze DNA Concentration

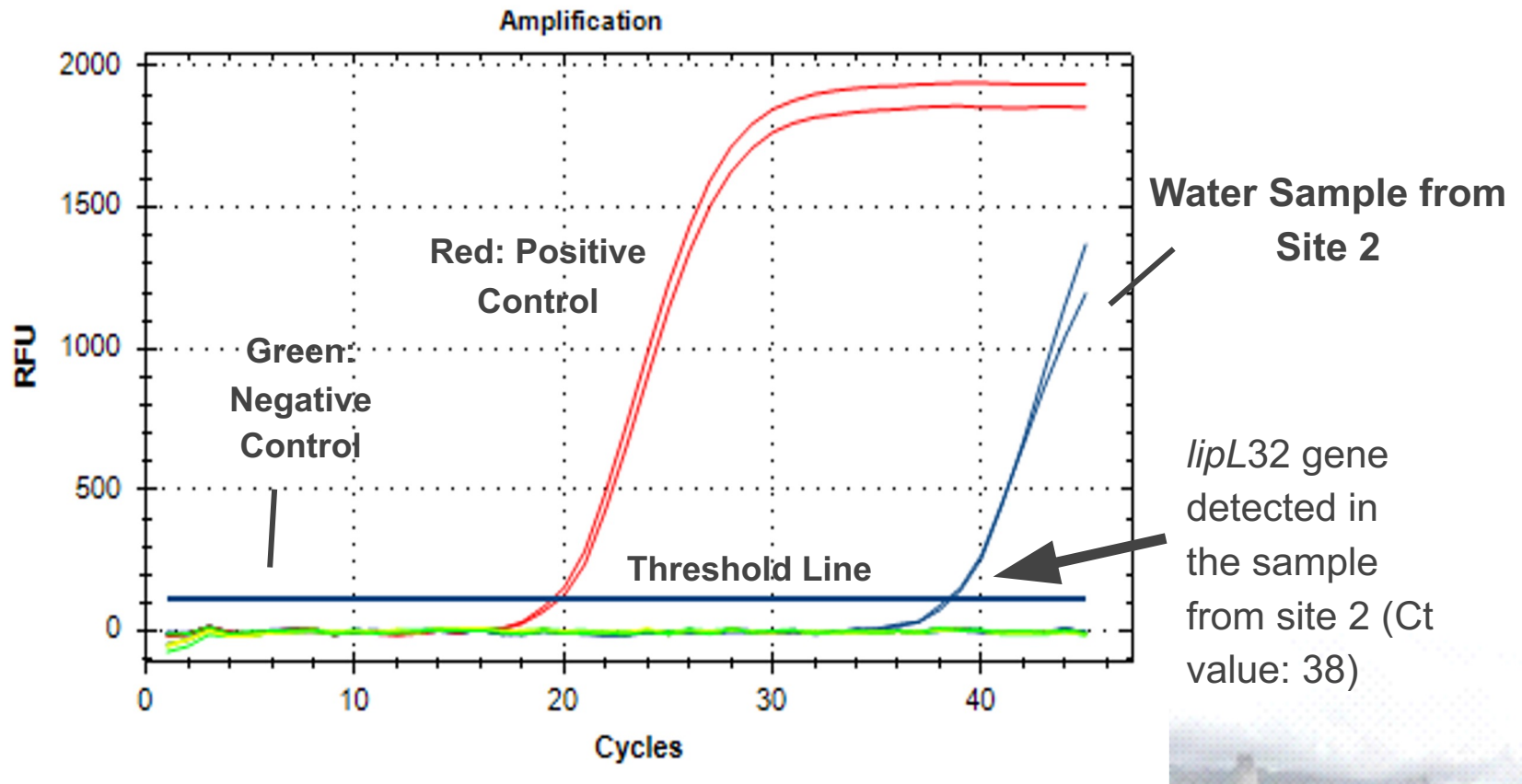
DNA samples were analyzed using a spectrophotometer



05 Quantitative PCR analysis for *lipL32* gene



Results



Results

DNA concentration and absorbance value:

Sample	DNA Conc. (ng/ μ L)	A260	A280	260/280	260/230
Site 1A	5.5	0.111	0.065	1.69	0.23
Site 1B	1	0.02	0.005	3.81	0.12
Site 2	5.9	0.118	0.053	2.21	0.27

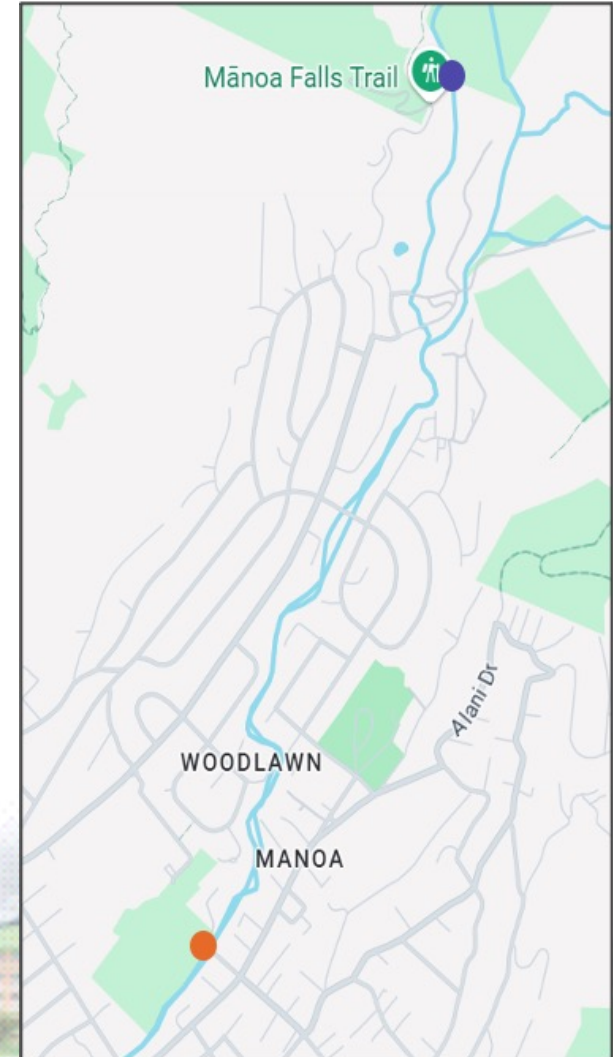
qPCR Results Summary:

Sample	Cq Value(s)	Summary Notes
Site 1B	NaN	No amplification detected (Negative)
Site 1A	NaN	No amplification detected (Negative)
Site 2	38.3, 38.5	Late amplification detected (Positive)
NEG	NaN	Non-pathogenic <i>Leptospira</i> DNA (<i>L. biflexa</i> serovar Patoc1), 14 ng DNA
NTC	NaN	No-template control (MIRD water) – no amplification
POS	19.37, 19.72	Pathogenic <i>Leptospira</i> DNA (<i>L. interrogans</i> serovar icterohemorrhage), 14 ng DNA

Conclusions

Is pathogenic *Leptospira* DNA present in the Mānoa Stream?

- Pathogenic *Leptospira* DNA was detected at one of the two sites sampled
- Presence of DNA may correlate with infectious pathogenic *Leptospira* bacteria in the streams
- Pathogenic *Leptospira* are not found at all locations tested in the stream
- Leptospirosis sign placement may need to be revised to reflect current *Leptospira* hazards



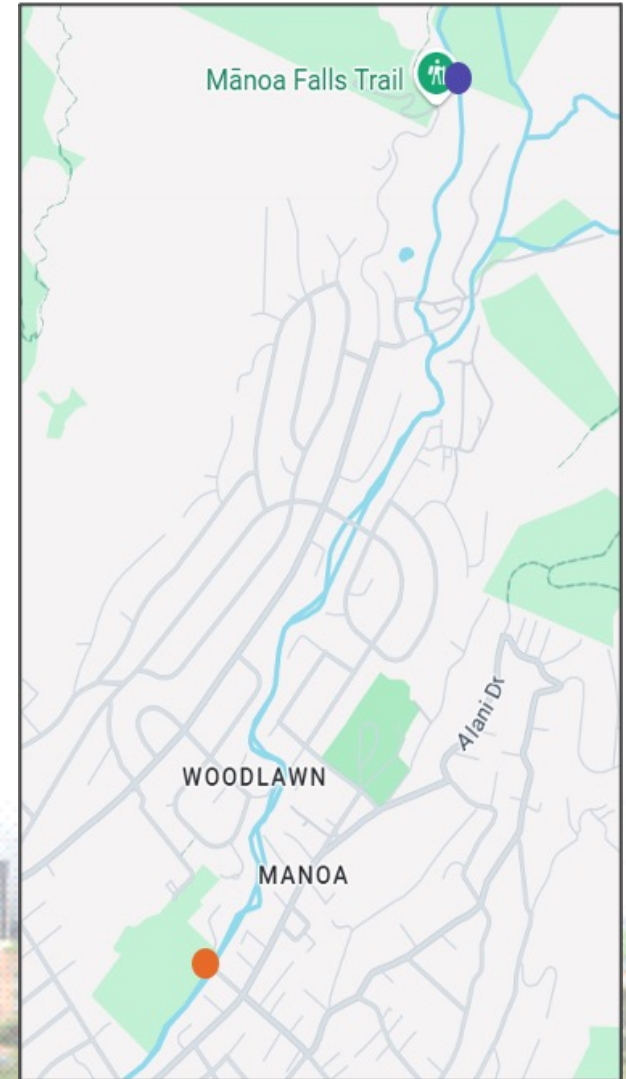
Future Directions

01 **Culture *Leptospira* from water samples**

Isolate *Leptospira* bacteria from the filter membrane

02 **Test additional water samples for pathogenic *Leptospira***

Collect water samples from the same locations during dry and rainy seasons, after storms, and at additional locations along the Mānoa stream



References

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