DETECTING THE SOURCE OF FECAL POLLUTION IN THE TILLAMOOK WATERSHED WITH A NEW MOLECULAR METHOD

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THE ISSUE

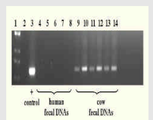
- Fecal contamination of aquatic environments affects Tillamook Bay, Oregon, and its surrounding watershed
- Usually the source of fecal contamination cannot be determined
- In order to assess human health risks and develop management plans for coastal and inland waters, it is necessary to know the sources of fecal contamination

BACKGROUND

- In the Tillamook Watershed, failing septic systems, sewage treatment plant overloads, wildlife populations, and runoff from agricultural sources are potential sources of fecal pollution that spreads pathogens, impacts the integrity of aquatic ecosystems and affects recreational and fisheries use of the waters.

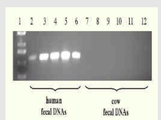
- Standard methods of measuring fecal pollution (growing fecal coliforms, *Escherichia coli*, or enterococci from water samples to estimate concentrations) do not distinguish between human and animal sources, and are time-consuming.

- At Oregon State University, we developed a method of fecal source tracking based on 16S rDNA markers from the Bacteroidetes group of fecal anaerobes. These bacteria are common in feces, diverse, and do not grow in aerobic environments. Detection by PCR circumvents the difficulty of growing anaerobic bacteria.



PCR with ruminant primer

Cow vs. human



PCR with human primer

- In a pilot study in the watershed, most of the samples that were positive were located in the bay and on two of the five major rivers that drain the Tillamook basin. These two rivers have the majority of on-site septic systems, and high concentrations of dairy farms.

APPROACH

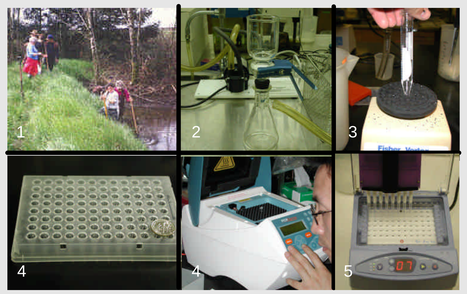
1. **COLLECT WATER SAMPLES** Tillamook Estuarine Project citizen volunteers collected water samples twice monthly from 30 sites throughout the watershed.

2. **FILTER** Samples were filtered to trap microorganisms. Filters were stored in guanidine thiocyanate buffer and shipped to OSU.

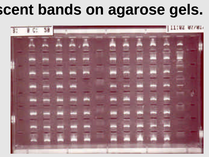
3. **EXTRACT DNA** At OSU, DNAs were extracted from the filters.

4. **PCR WITH SOURCE-SPECIFIC PRIMERS** 96 PCR reactions at a time were performed in microtiter plates.

5. **EXAMINE FOR POSITIVES BY GEL ELECTROPHORESIS**

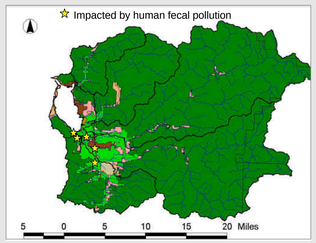


6. General, ruminant and human fecal pollution in each water sample was detected as fluorescent bands on agarose gels.

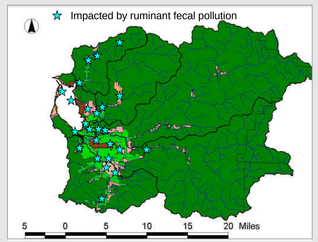


RESULTS

- A few sites (5 out of 30) were heavily impacted by human fecal pollution. Human fecal pollution occurred in over 70% of a year's samples at these sites.



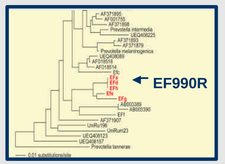
- The majority of sites (26 out of 30) were heavily impacted by ruminant fecal pollution. Ruminant fecal pollution occurred in over 70% of a year's samples at these sites.



- Although the most likely source of the widespread ruminant fecal pollution is dairy cows, elk herds are also present.

We used subtractive hybridization in microtiter dishes to develop a new primer that distinguishes between cow and elk fecal pollution. In this technique, target DNA is allowed to hybridize to subtractor DNA fixed in a microtiter well. Unique target sequences are left unhybridized in solution.

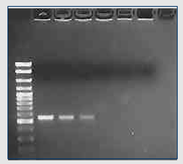
- Subtractor: amplified Bacteroidetes 16S and 23S fecal rDNAs from cows and humans
- Target: amplified Bacteroidetes 16S and 23S fecal rDNAs from elks
- Remaining unhybridized DNA (lanes 2-5) was cloned and sequenced



- We aligned the unique sequences (shown in red) with known sequences, and used them to design an elk-specific PCR primer, EF990R

- After optimization, the new primer specifically amplified elk fecal DNAs, and did not amplify cow fecal DNAs.

- We will use the new primer to distinguish between wild and domestic ruminants in the samples that were positive for ruminant fecal pollution



We can use Real-time Quantitative PCR (Q-PCR) to calculate the proportion of human fecal pollution in the total:

- Define X as the ratio of a human PCR marker to the general Bacteroidetes marker in sewage
- the amount of human marker in a water sample can be divided by X to estimate the proportion of general Bacteroidetes marker in the sample attributed to human fecal contamination

CONCLUSIONS

- Amplification of genetic markers from Bacteroidetes provides a rapid, sensitive, inexpensive and accurate method of fecal source discrimination, which has allowed us to identify areas impacted by either ruminant or human fecal pollution.
- Ongoing research will differentiate between domestic and wild ruminant pollution, add more species, and estimate loading from each source.
- OSU researchers benefited by obtaining high-quality samples for ongoing source-tracking research.
- Tillamook Bay citizens and scientists benefited by obtaining state-of-the-art source tracking data to identify areas for mitigation.