

Uncertainty Issues Relating to Detection of Non-Human Indicators

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State of the Science: Fecal Source Identification and Associated Risk Assessment Tools



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- 1. Background
- 2. Sources of Uncertainty
- 3. Some Observations
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Background: Estimated non-human animal population sizes in California

Animal	Group	Population Size Estimate				
Cats	Companion	8,967,756				
Dogs	Companion	7,948,978				
Cattle	Agricultural	5,350,000				
Sheep	Agricultural	570,000				
Wild Canids	Wildlife	500,000				
Deer	Wildlife	445,000				
Sea Lions	Wildlife	334,000				
Gulls	Wildlife	46,800*				
Wild Felids	Wildlife	5,000				
Goats	Agriculture	3,500				
Sea Otters	Wildlife	2,711				

* Estimate from San Francisco Bay only



Background: A microbial source tracking solution



SOLUTION... Method designed to collect, isolate, and detect a fecal pollution source from an environmental sample.

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Background: Why should microbial source tracking work?

Intestinal microbes of animal groups are expected to be different:

- Gut conditions
 - Temperature
 - Diet
 - Digestive physiology
- Natural selection
 - Space
 - Nutrients

Microbe: a microscopic organism, especially a bacterium, virus, or fungus *Oxford Dictionary*









Background: Currently available nonhuman microbial source tracking methods*

Trends in SIPP Study:

- Most methods available for ruminant/cattle
- No methods available for many animals
- Little known about performance compared to human-associated methods

Animal	Number of Methods*
Cattle	9
Pig	3
Gull	2
Dog	2
Horse	1
Sheep	1

* Data from SIPP study PCR-based methods only



Sources of Uncertainty





Host Biology Uncertainty: Indicator distribution in non-target animals



- High specificity can still be problematic
- Especially when indicator quantities are equivalent to target host values for key animal groups
- Method selection may vary from one site to another



Host Biology Uncertainty: Indicator distribution in target animals

	All	By Cattle Population										
Assay		Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	Herd 7	Herd 8	Herd 9	Herd 10	Herd 11
CF128	85%	96%	92%	70%	33%	100%	100%	97%	100%	100%	100%	100%
CF193	68%	76%	0%	0%	10%	100%	100%	97%	100%	100%	100%	100%
Bac2	54%	0%	0%	0%	0%	90%	100%	80%	87%	100%	100%	87%
Bac3	69%	16%	80%	0%	0%	90%	100%	100%	100%	100%	100%	100%

Test quantity = 1 ng total DNA/reaction

- Uneven distribution across 11 populations
- Frequency of detection ranges from 0% to 100%





Host Biology Uncertainty: Influence of animal diet

 Fecal bacteria population diversity and indicator shedding are linked to animal diet

- Experiment design:
 - 30 adult cow fecal samples
 - 6 different populations
 - 3 animal diets
 - 454 pyrosequencing



Squares represent individual fecal samples; projections indicate individual OTUs; diameter of projection denotes abundance within a sample; length of projection indicates abundance across samples.



Host Biology Uncertainty: Influence of animal age on indicator detection

- Indicator shedding linked to animal age
- Adult levels remain consistent



Experiment design: 31 cow/calf pairings Sampled (March - October 2011)

7 sampling dates



Observations: Some Food for Thought

- Host biology uncertainties dramatically influence data interpretation
- Must be familiar with local animal groups
- Likely that these trends exist for many animal groups
- Currently big gaps in the non-human indicator "tool box"











- Prioritization of animal sources for future indicator method development (deer, rodents, cats?)
- Characterization of indicator distributions for high priority animals
- Improve pathogen data for high priority animal sources
- Define and prioritize source tracking applications (beach risk assessment, TMDL, BMP evaluations)
- Development of data interpretation algorithms that account for uncertainties and particular application
- Continue performance characterization of emerging technologies
- Field application case studies



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