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# Innovative Methods Supporting Local Water Quality Decision Making: Microbial Source Tracking

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# Water Quality Problems in AZ

- Arizona Department of Environmental Quality (ADEQ) has about 124 lakes and stream reaches listed as “impaired”.
- More than \$12,000,000 in grants since 2000.
- Grant projects have resulted in delisting one part of one impaired stream (Nutrioso Creek).

# ADEQ Targeted Watershed Grant Program

- Water Quality Improvement Grant Objective
  - focus on on-the-ground non-point source priority projects, so that in the near future an impaired water will meet water quality standards.
- Targeted plans (WIP) are developed for watershed drainage areas contributing non-point source pollutant loadings that are causing impairments.



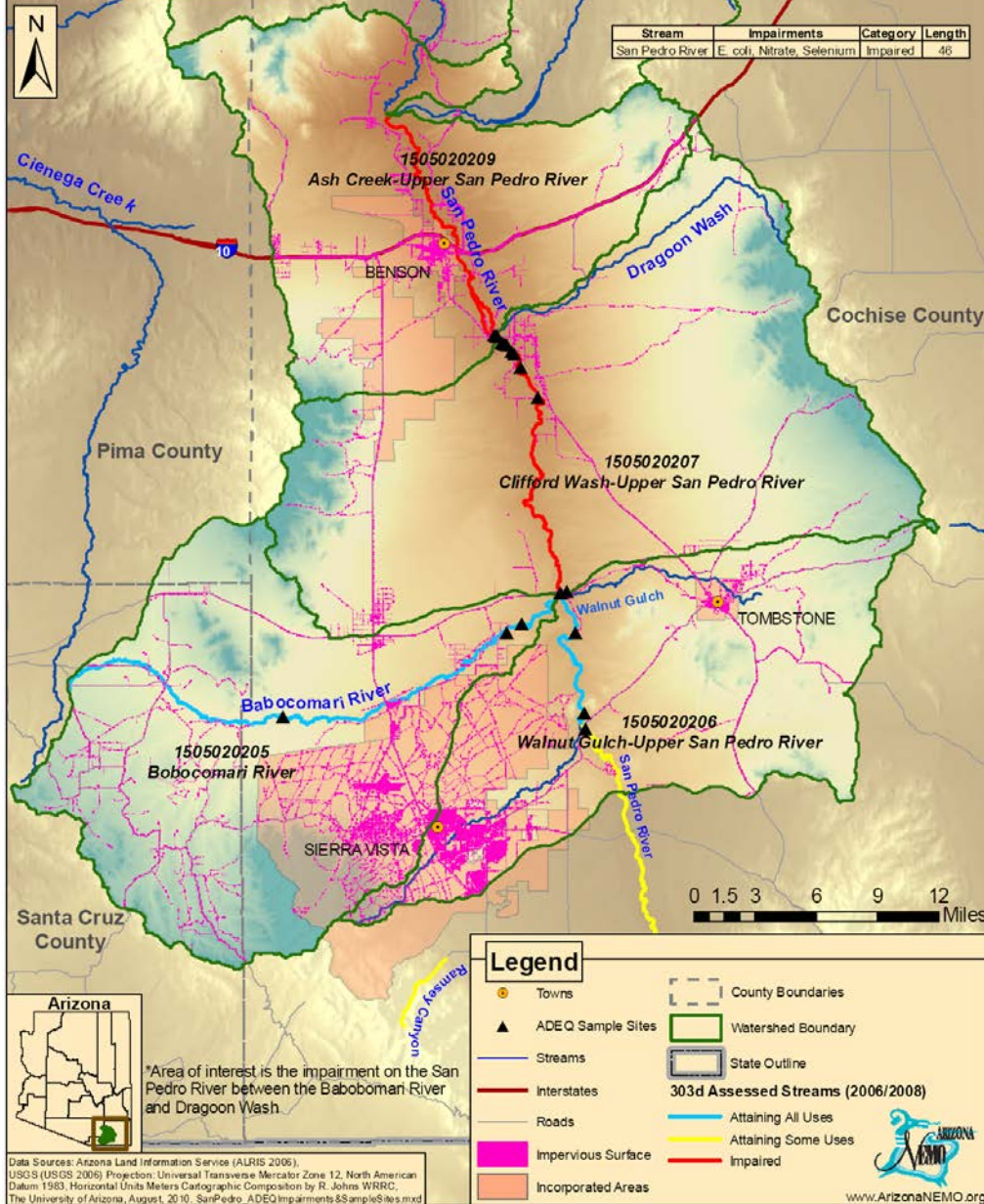
# Where does UA fit in?

- ADEQ's Grant Program has contracted with the University of Arizona's Cooperative Extension to provide technical support and training opportunities.
- Technical support in the development of watershed based plans, such as:
  - modeling
  - GIS assisted mapping
  - Best Management Practices (BMPs)
  - Volunteer sampling training
  - water quality testing relating to microbial source tracking.





# San Pedro River Targeted Watershed



- San Pedro River
- Granite Creek
- Oak Creek
- San Francisco River
- Santa Cruz River
- Pollutant of concern: *E.coli* bacteria



# Contamination of surface water



**Wastewater**



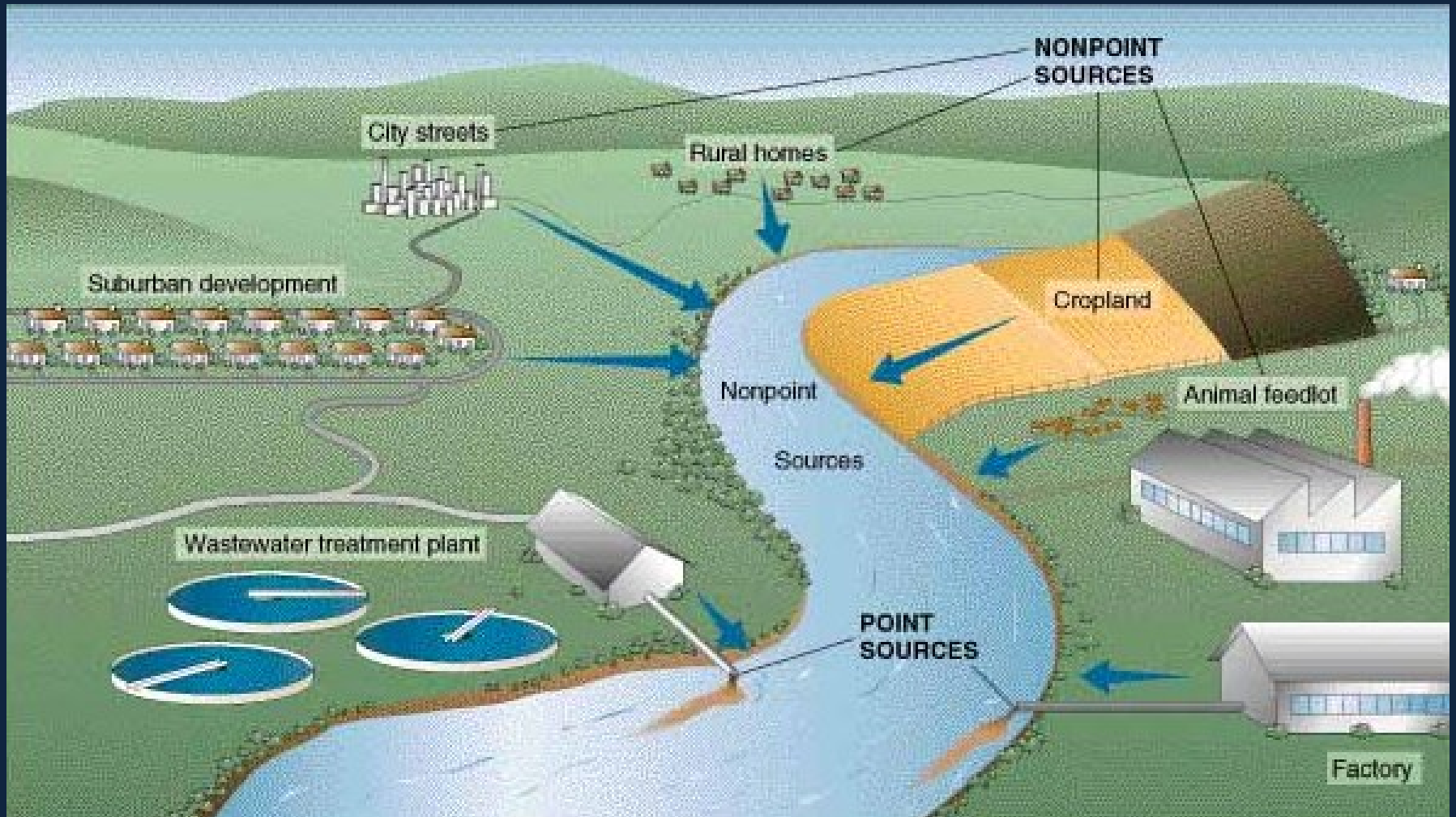
**Recreation**



**Domestic &  
Wildlife**

There's *E.coli* in the water, but where did it come from?

# NON-POINT & POINT SOURCE POLLUTION



# Targeted Non-Point Source Pollution Projects

- Work to identify **non-point source pollution** contributions around the state.
- These watersheds that are polluted by non-point source pollution are **targeted watersheds**
- 303 (d) listed waters are in targeted watersheds and funding can be given for improvement projects to reduce pollution



# Water Quality

- **Water quality is a term used to describe the chemical, biological, and physical characteristics of water.**
- **Water quality is not simply "good" or "bad", but usually is applied to its **use**.**
- **For example, drinking water quality will be different than recreational water quality for swimming and/or fishing, or recycled water quality used for irrigation, etc...**

# Types of Measurements to Determine Water Quality

- **Chemical**

Metals, organic compounds, pesticides, herbicides, nutrients, etc.

- **Physical**

Temperature, pH, turbidity

- **Microbial**

Bacteria, viruses and parasites



# Samples and Standards

- Water samples that **exceed the 235 MPN/100mL *E.coli* standard for a single sample** are considered unsafe for swimming or irrigating
- **High turbidity** can be a visual marker for potentially high *E.coli*, especially after storm flow.
- **Water temperature** above 37C can allow for *E.coli* to remain alive or proliferate in rivers



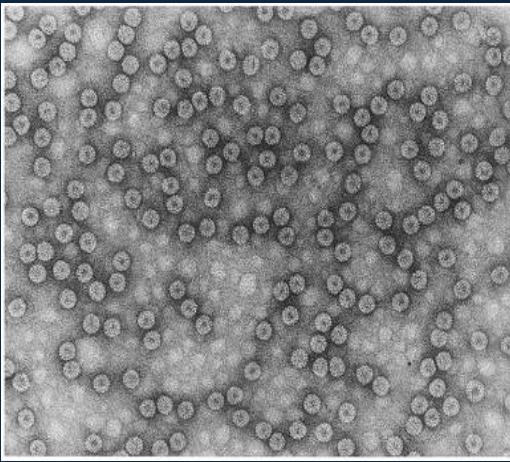
# Organic Pollution

- Human sewage, animal waste, and plant residue also contain organic material, which can pollute water.
- Bacteria in the water decompose the organic material, producing additional nutrients for plant growth.
- This can cause further decreases in oxygen content in the water.
- Human and animal wastes can also carry harmful bacteria and viruses that can spread diseases caused by **enteric pathogens**.

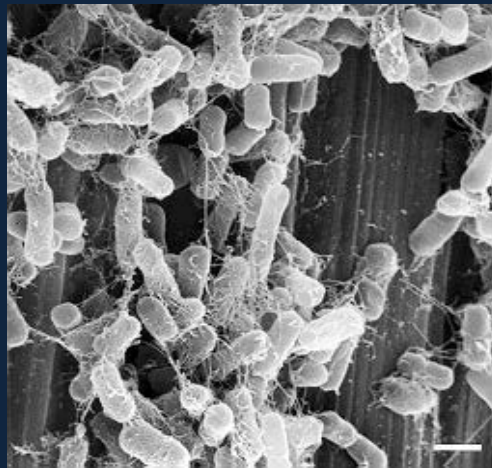


# Types of Waterborne Pathogens

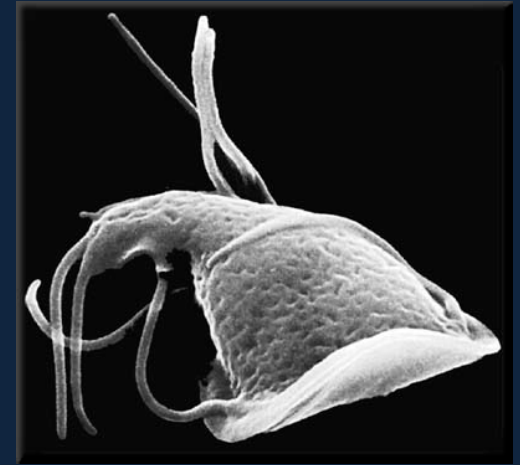
## Viruses



## Bacteria

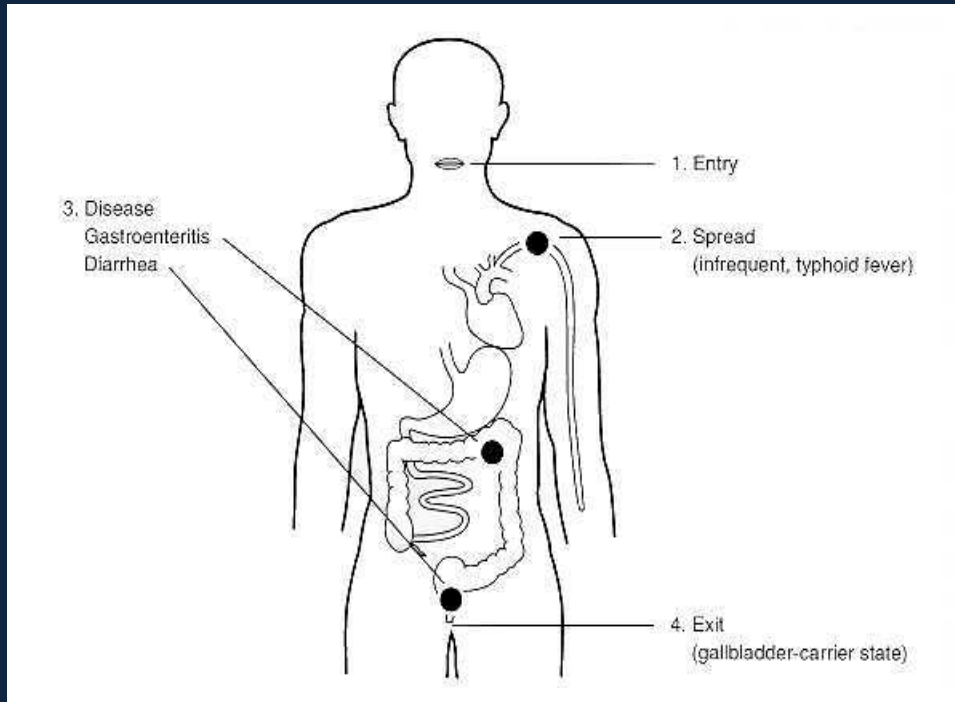


## Parasites



A pathogen is something that causes disease

# Enteric Pathogens



- Exposure is via ingestion
- Primary site of infection is gastrointestinal tract
- Gastroenteritis symptoms
  - nausea
  - vomiting
  - diarrhea
  - fever
- May spread to other sites (blood, liver, nervous system)
- Shed in fecal material
- “Fecal-oral” route of transmission

# Microorganisms

- **Examples:**
  - **Bacteria – *Escherichia coli (E. coli)*, *Salmonella***
  - **Viruses – Rotavirus, Adenovirus**
  - **Parasites – *Cryptosporidium*, *Giardia***
- **Pathogens cause disease. These microorganisms can also be shed in the feces of humans and animals.**

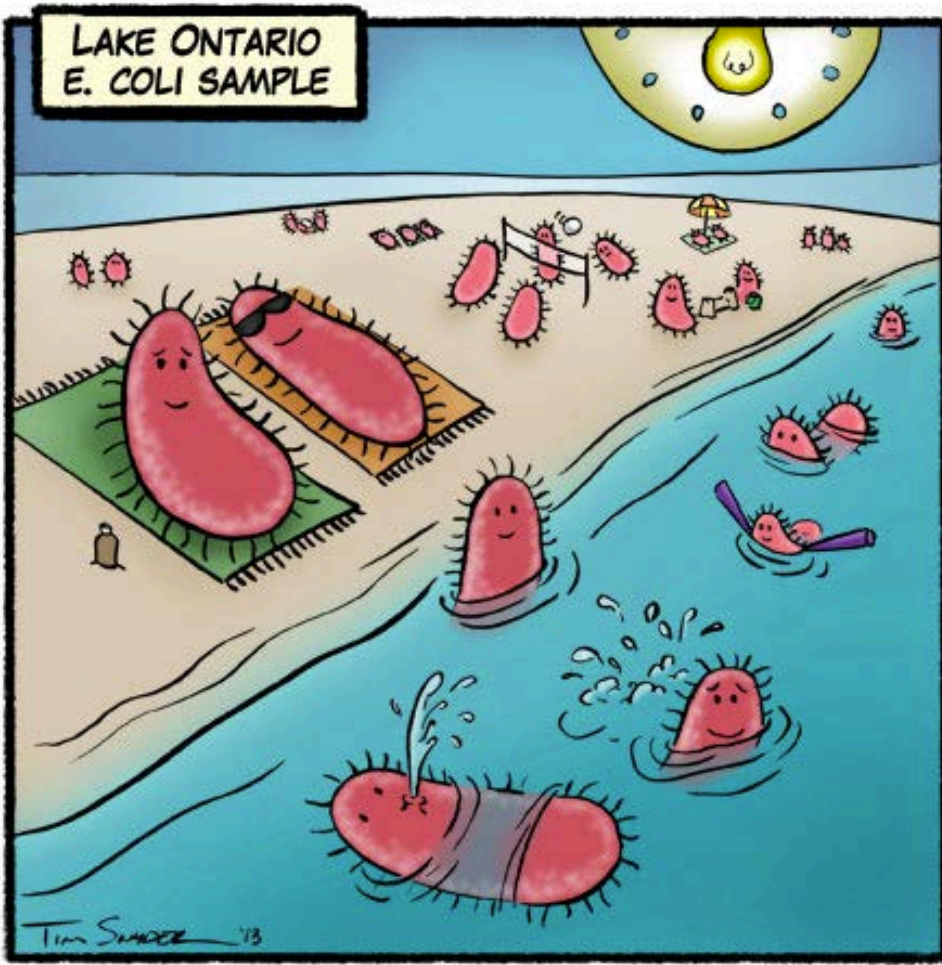
How can we tell if surface water has been contaminated by feces?

Indicator Bacteria

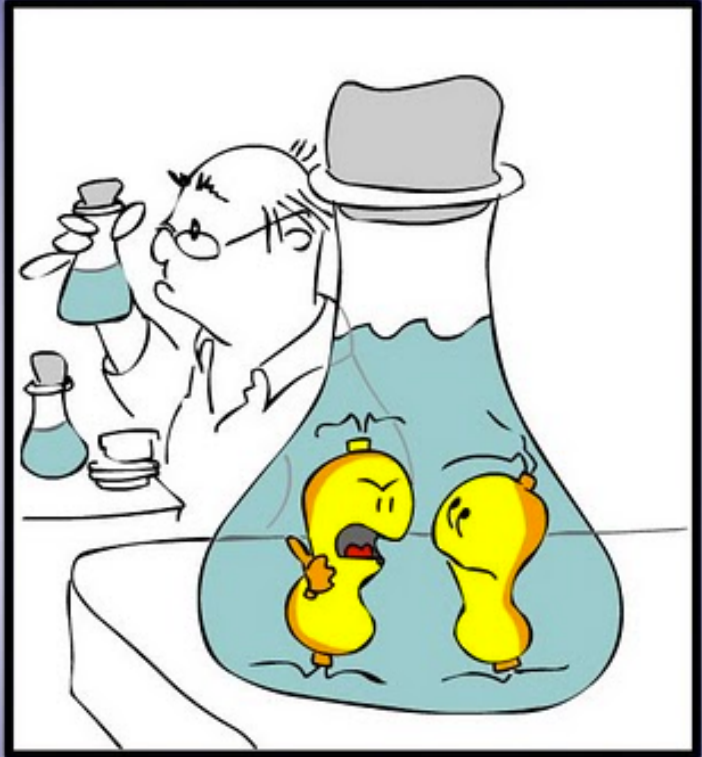


# Indicator Bacteria

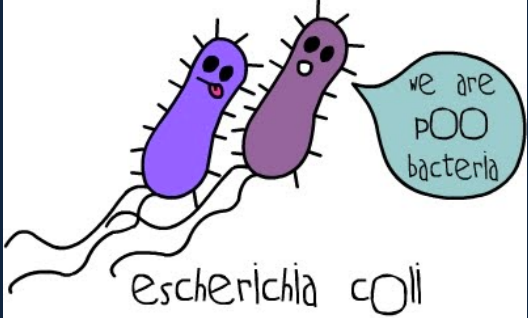
- Total coliform bacteria
- Fecal coliform bacteria
- *E.coli* ( *Escherichia coli* )
- Indicates fecal contamination if fecal coliforms are present; could also indicate pathogens are present; used as a water quality indicator.
- ***E. coli* is used as a national standard for drinking and recreational water quality.**

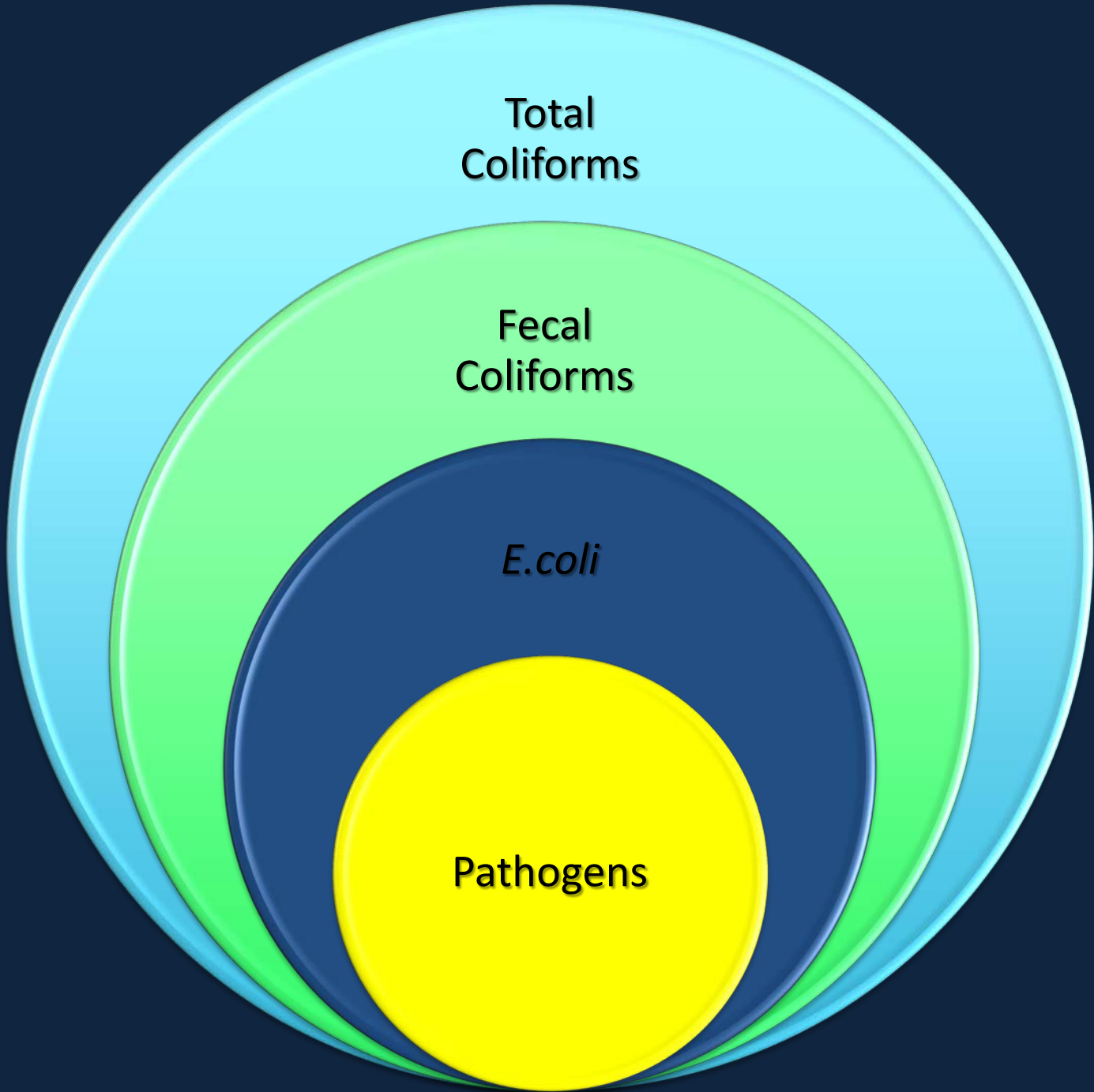


www.tonup.ca



I'M FED UP WITH THIS GUY -  
LET'S BECOME PATHOGENIC





Total  
Coliforms

Fecal  
Coliforms

*E.coli*

Pathogens

# Rules for Fecal Indicator Bacteria

- Bacteria from feces of warm-blooded animals
- Present in higher numbers than pathogens
- Nonpathogenic
- Do not persist longer than the pathogen in the environment

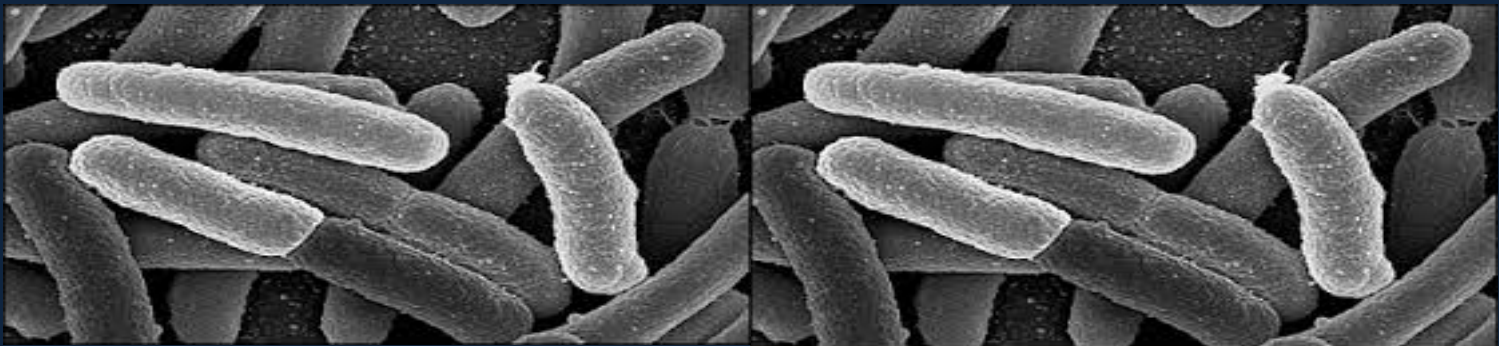


# Why not sample for pathogens?

- Few laboratories have the capacity
- It's expensive
- Takes a long time for analysis
- Requires a large volume of water
- Most tests identify only one pathogen
- They are difficult to isolate and identify

# What the Heck is *E. coli* ?

- *Escherichia coli* (*E.coli*) is a bacterium naturally found in the intestines and the feces of warm-blooded animals.
- Commonly used as an indicator of **fecal** pollution of water.
- Many different types of *E. coli*, most harmless, but some may cause illness (e.g. hamburger restaurant outbreaks caused by *E. coli* O157:H7)



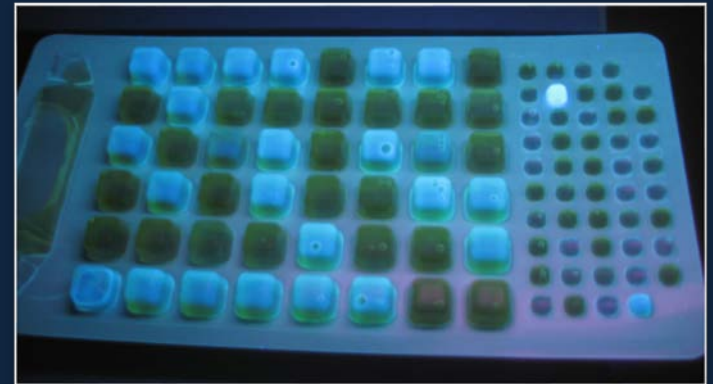
# *E.coli* are used as indicators because they:

- Indicate fecal contamination
- Suggest the presence of pathogens
- Are easy to collect and analyze
- Are relatively safe to handle and generally harmless

# What Does *E.coli* look like?

- Cultural Methods

- Petri Dish

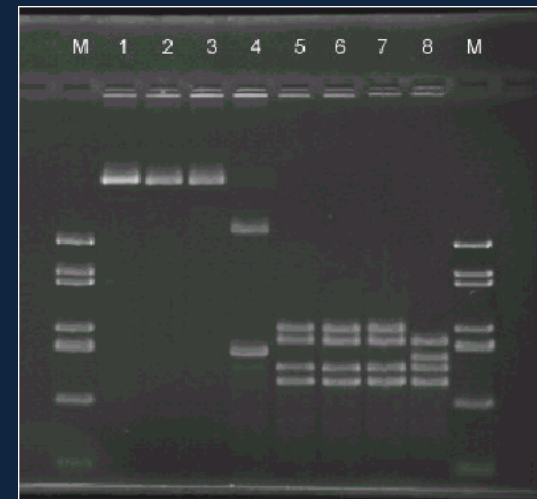


IDEXX QUANTI TRAY (MPN/100mL)

- Molecular Methods

- DNA

- MST



DNA BANDS on ELECTROPHORESIS GEL

# Alternatives to *E.coli*: *Bacteroides*

- Bacteria belonging to the genus *Bacteroides* have been suggested as alternative fecal indicators to *E.coli* or fecal coliforms
- They make up a significant portion of the fecal bacterial population
- Have little potential for re-growth in the environment
- Have a high degree of host specificity that likely reflects differences in host animal digestive systems

# Source Tracking

Methodologies aimed at identifying dominant sources of contamination in environmental samples

Chemical

Microbial

Residual chemicals unique to a source  
(caffeine)

Phenotypic patterns  
(antibiotic resistance)

Molecular markers



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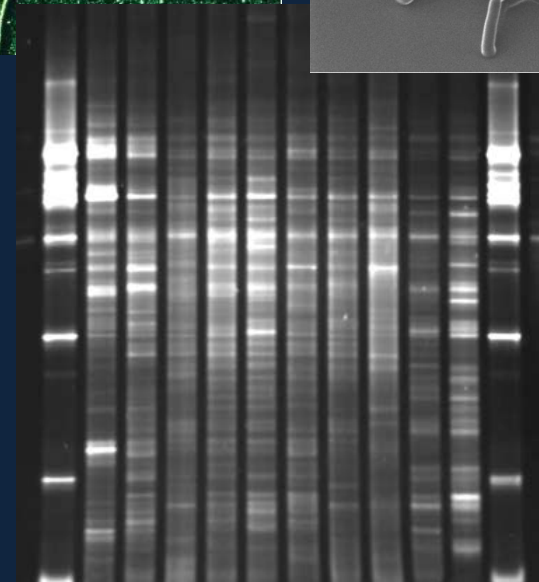
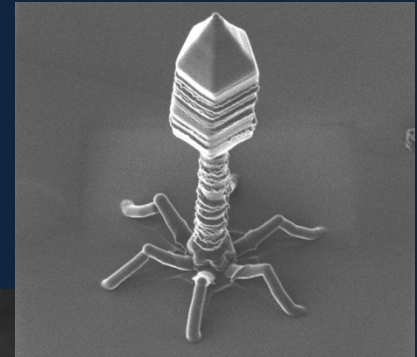
# Source Tracking

## Using Molecular Microbial Markers

Fecal organism carries a molecular “signature” that is unique to its source

Biochemical tracers;  
Fingerprinting techniques

Host-specific 16S rDNA  
genetic markers



# Source Tracking Municipal Wastewater Retention Basins

Filled with tertiary-treated  
recycled municipal water

*E. coli* in pond and  
irrigation water averaging >  
50 CFU 100 mL<sup>-1</sup>

Do we have regrowth of  
human fecal bacteria?



# Source Tracking Using Host-Specific *Bacteroides* Molecular Markers

Exclusively found in feces, rumens, and other cavities of humans and other animals

## THE PROS

Strict anaerobes (limited potential for growth in the environment)

Extremely diverse (~25% of total bacteria in feces)

Host-specific genetic markers can be used to evaluate fecal pollution

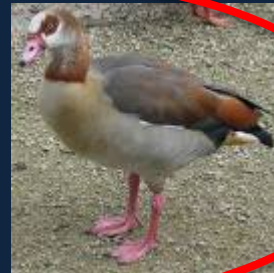


# Source Tracking Using Host-Specific *Bacteroides* Molecular Markers

Exclusively found in feces, rumens, and other cavities of humans and other animals

THE CONS: A new science

Misinformation abounds:  
“Exclusively in the guts of warm-blooded animals” (2000)

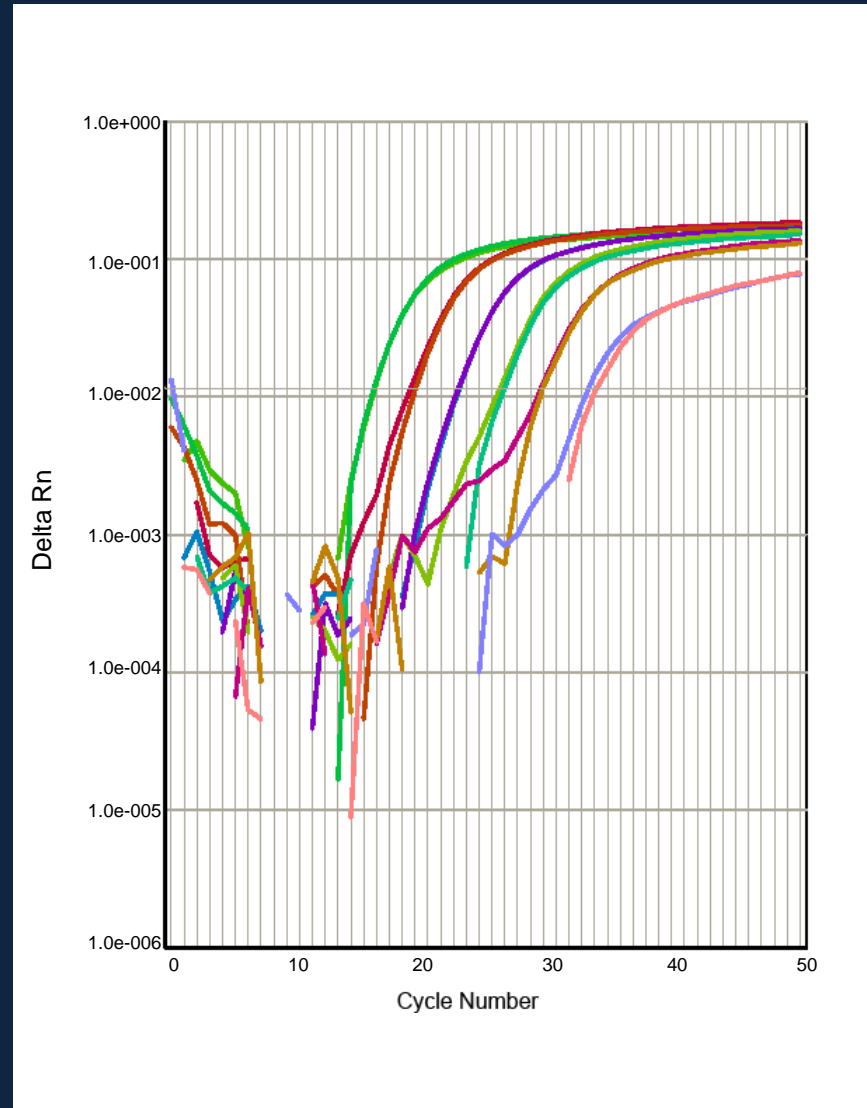


# Source Tracking Pacana Park

Use quantitative PCR to quantify human-specific *Bacteroides* molecular markers in pond water

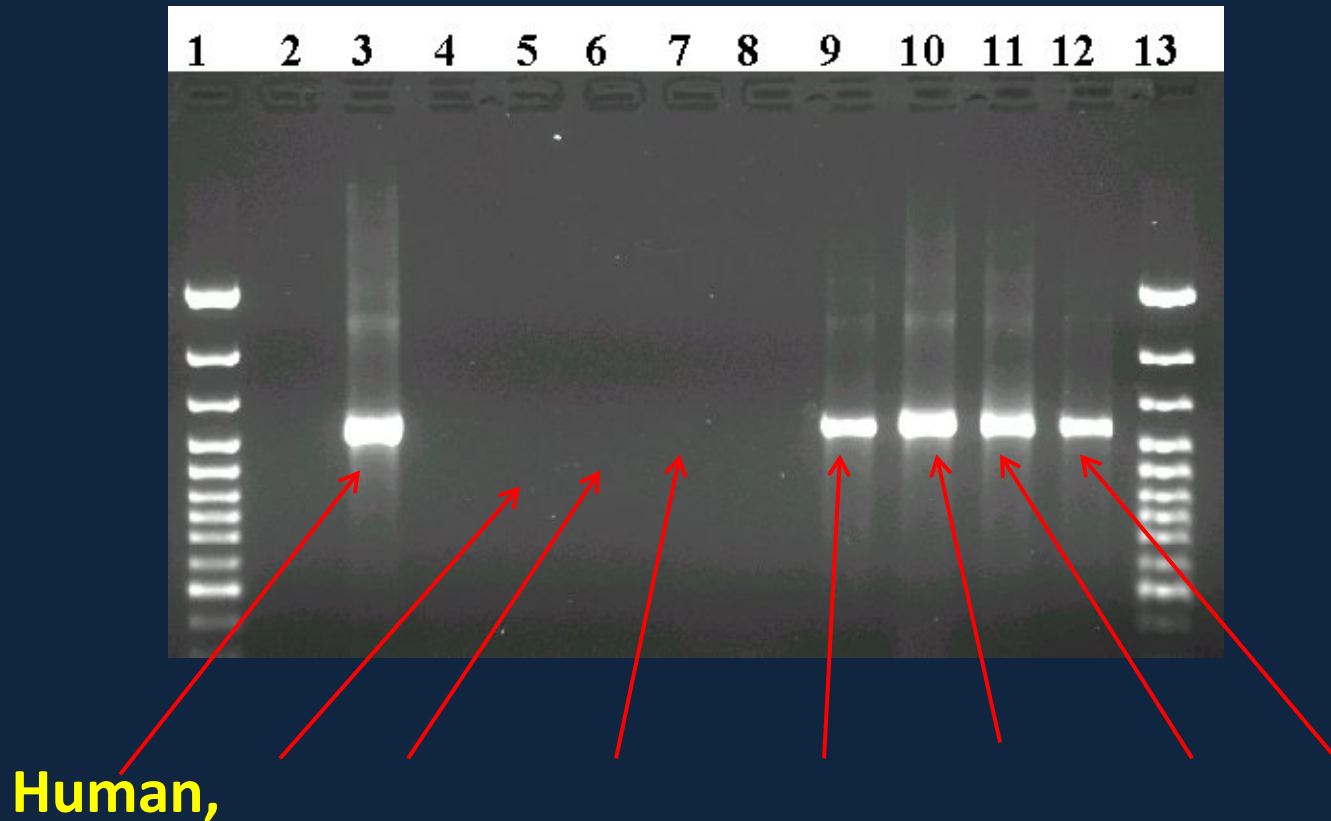
Over 6 months, human-specific markers averaged 4500  $100 \text{ mL}^{-1}$  of water

Did we identify human fecal contamination?

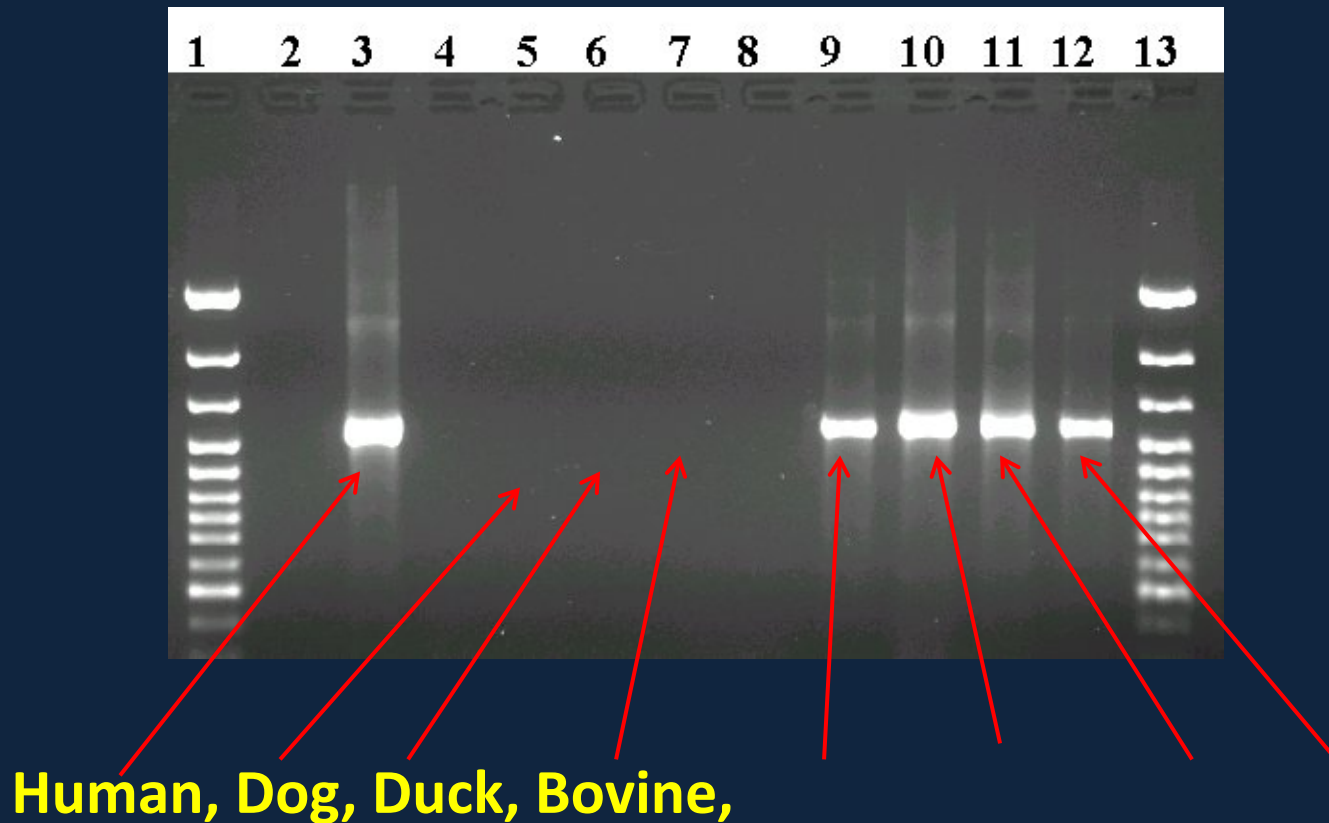




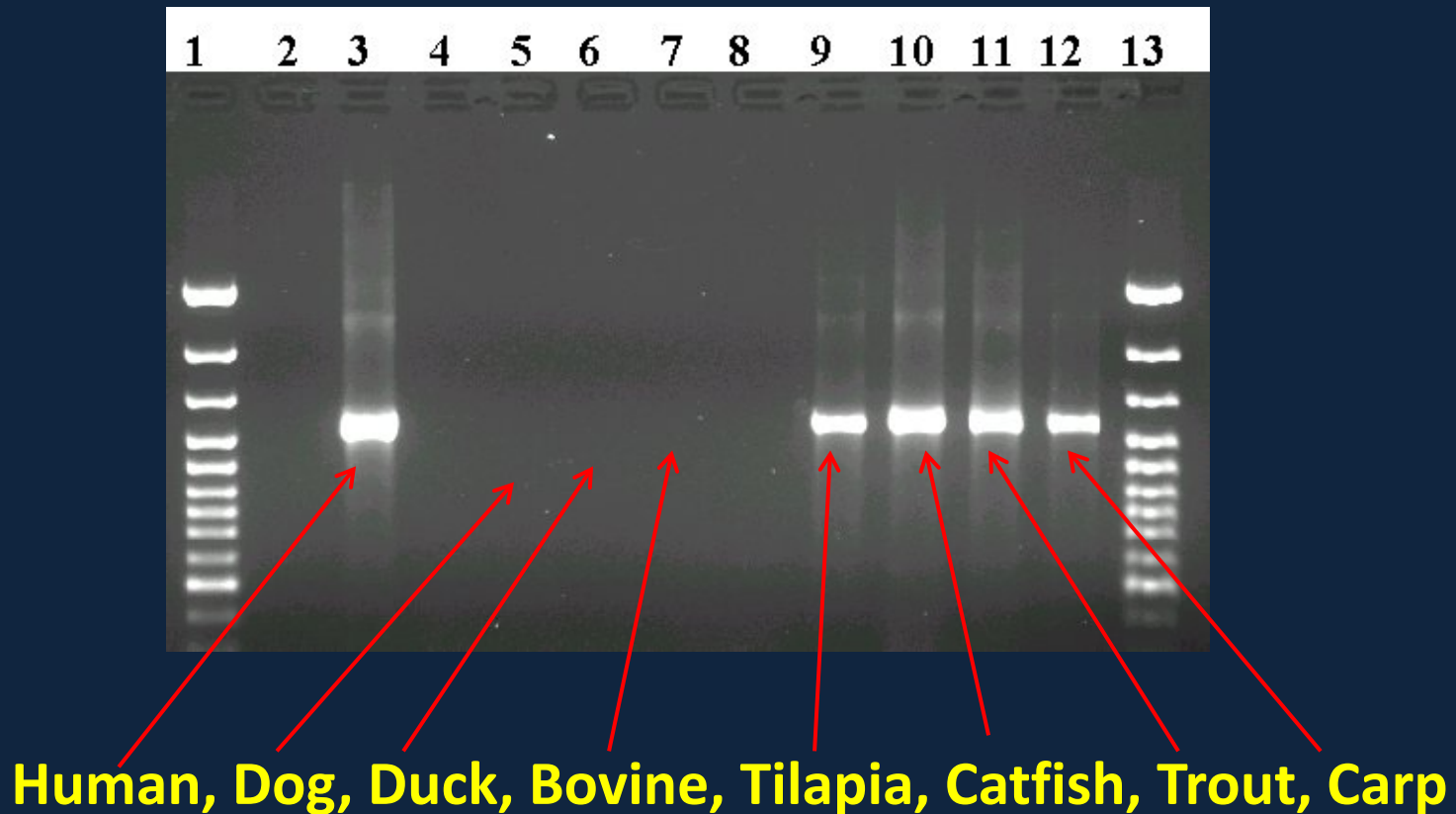
# Validation of PCR Results with Known Fecal Sources



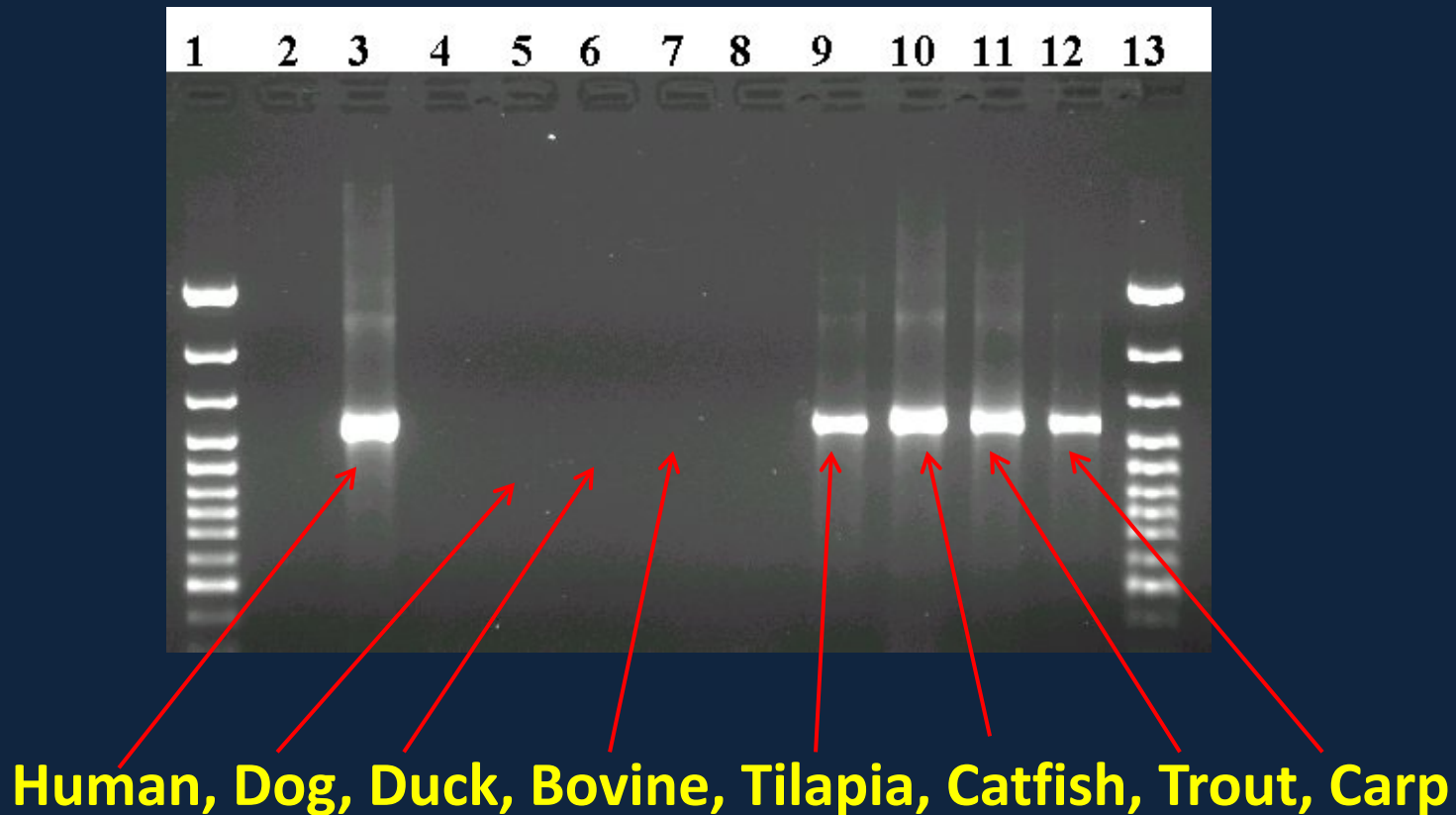
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# Validation of PCR Results with Known Fecal Sources



# Validation of PCR Results with Known Fecal Sources



Affects conclusions of dozens of published source tracking studies performed in water containing fish

# Follow-Up Work

3 of “human-specific”  
*Bacteroides* assays cross-  
amplified with fish feces\*

Source tracking is an  
emerging science: known  
fecal inputs must be tested  
for non-target markers in  
ANY molecular source  
tracking study



\*McLain et al. (2009); Kabiri-Badr et al. (2014)

# Source Tracking in the Yucatan

Groundwater-dependent system

Riviera Maya: visited by more than 1.7 million tourists per year

Direct injection of untreated waste to groundwater

Severe water quality issues





# Source Tracking in the Yucatan



# Source Tracking in the Yucatan





# Sources of Water Quality Degradation





# Source Tracking in the Yucatan

Source tracking using  
antibiotic resistance

Erythromycin resistance  
significantly higher in  
bacteria isolated from  
“local” sources

Screening of chemical  
constituents

Sucralose



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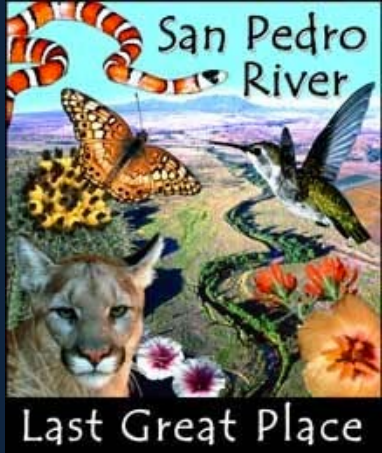
Sucralose

Viagra





# Working Together for Clean Water





# THANK YOU



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