

# **HOW TO MONITOR FOR FECAL BACTERIA**

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# WHY MONITOR FOR FECAL BACTERIA

- Fecal bacteria is the largest impairment source of streams in Virginia; currently at 9,154 miles
- Affects recreation, shellfish, and animal uses
- Waters with high fecal bacteria usually also have high sediment and nutrient levels
- High levels of fecal bacteria and sewage in waterbodies increase the risk of illness



Now on YouTube! Search 'Attack of the E. coli' or go to

[www.youtube.com/watch?v=fyY6YF9xtzc](http://www.youtube.com/watch?v=fyY6YF9xtzc)



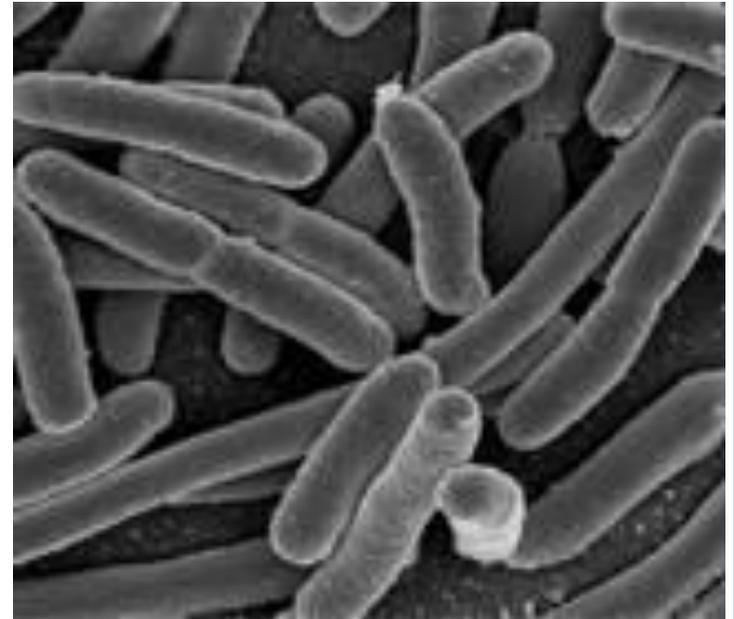
## BEFORE WE GO FURTHER...

- Culture tests have results reported in CFU or MPN
- CFU or Colony Forming Units is the number of bacteria colonies that formed from the original bacteria in the sample
- MPN or Most Probable Number is a statistical estimate of the number of bacteria that was present in the sample
- Except for shellfish standards, Virginia Water Quality Standards only reference values by CFU
  - MPN data can still be used to gauge water quality
  - MPN data compares well with CFU results if using a large number of ‘tubes’ such as the QuantiTray 2000 used in the Colilert™ method.



# ESCHERICHIA COLI (E. COLI)

- Gram negative, rod shaped, facultative anaerobic bacteria
- Normally found in the digestive tracks of warm blooded animals
- Currently monitored in fresh waterbodies
  - Samples with  $>235$  E. coli or an average  $>126$  per 100 ml sample indicates excessive bacteria levels



# FECAL COLIFORM

- Family of coliform bacteria species only found in the digestive tract of warm blooded animals
  - E. coli and other species are members
  - Since 2002, no longer used for freshwater recreation (e.g. swimming)
  - Still used by VDH to determine closure of shellfish beds. Average fecal coliform levels must be less than 14 and single maximum readings no more than 49 MPN or 31 CFU



# ENTEROCOCCUS

- Gram positive, spherical shaped, facultative anaerobic bacteria
- Normally found in the digestive tracks of warm blooded animals
- Currently monitored in saltwater (beaches)
  - Samples with  $>10^4$  E. coil or an average  $>35$  per 100 ml sample indicates excessive bacteria levels



# METHODS TO MONITOR FOR FECAL BACTERIA

- Culturing of samples
  - Presence/Absence
  - Coliscan Eaygel
  - Petriflm
  - Colilert/Enterolert
  - Membrane Filtration
  - Multiple Tube Method



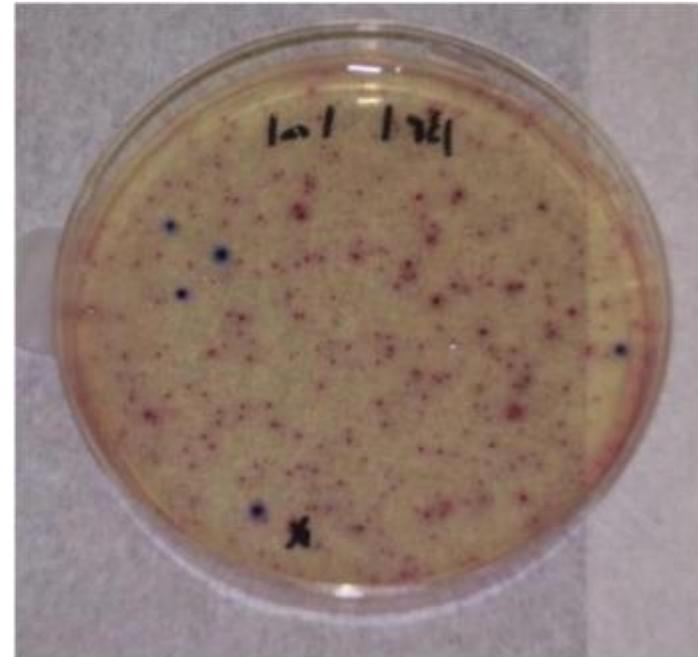
# PRESENCE/ABSENCE

- Simplest culturing method
- Sample placed in a bottle containing nutrient media
- Color change/glow under UV or similar reaction indicates presence of the bacteria
- Results in 24 hours
- Normally used to test drinking water quality as no fecal bacteria should be present



# COLISCAN EASYGEL

- An inexpensive and easy to use test for E. coli
- Does not require a laboratory
- Tests 1 to 5 ml of sample
- Results in 24 to 48 hours
- E. coli appear dark blue to purple
- Results are in CFU



1 ml sample with 5 E. coli colonies



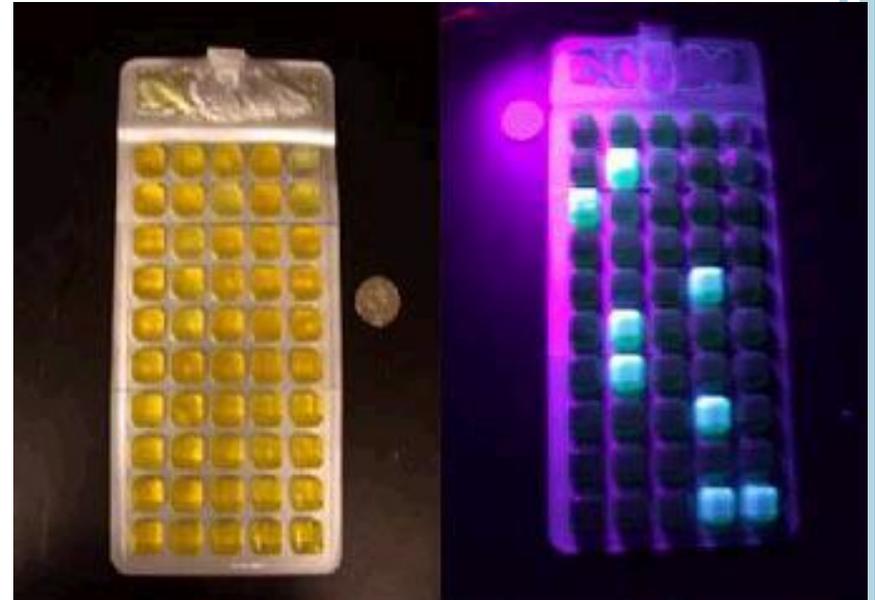
# PETRIFILM

- Non-laboratory based test to detect *E. coli* bacteria
- Tests 1 ml of sample but can be coupled with membrane filtration to test 100 ml
- *E. coli* appear dark blue with gas bubbles
- Results are in CFU



# COLILERT/ENTEROLERT

- Most popular lab based method due to relative low cost and ease of use
- Results in 18 to 24 hours
- E. coli glow under a UV light and have a yellow color
- Enterococcus method (for salt water) glows under a UV light but no color change
- Results in MPN



# MEMBRANE FILTRATION

- Filter sample through a fine pore (~0.6  $\mu\text{m}$ ) filter
- Tests up to 100 ml of sample
- Results in 18 to 24 hours
- Colony color depends on nutrient media
- Results reported as CFU



# MULTIPLE TUBE METHOD

- Not used as much due to introduction of Colilert/Enterolert
- Samples inoculate multiple test tubes of media/auger
- Results in 3 to 5 days
- Number of test tubes with a positive reaction determines the MPN result



# SUMMARY

Parameter	Presence/ Absence	Coliscan Easygel	Petrifilm	Colilert/ Enterolert	Membrane Filtration	Multiple Tube
Lab required	No	No	No	Yes	Yes	Yes
Min detection limit	0	<20	<100	0	0	0
Max detection limit	1	>6000	>6000	>10,000	>10,000	>10,000
Unit of measurement	N/A	CFU	CFU	MPN	CFU	MPN
Consumable cost	\$6.50	\$2.50	\$3.00	\$6.50	\$2.50	\$2.00
Equipment cost	\$0	\$100	\$1000	\$15,000	\$25,000	\$25,000
Time spent per sample	1 min	1 min	1 min	5 min	>10 min	>10 min
Incubation time (hrs)	24	24 to 48	24	18 to 24	18 to 24	72 to 120
DEQ Level Status	I	II	II	III	III	III





**Exercise Time**



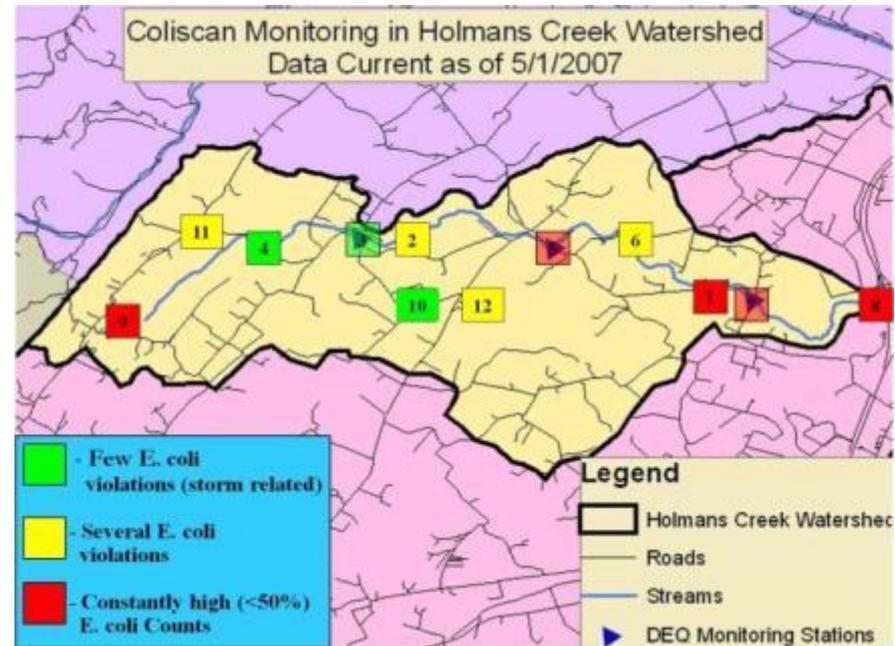
# EFFECTIVE MONITORING STRATEGIES

- “Shotgun” method
- Targeted source identification/verification
- Carpet sampling (AKA “B-52” method)
- Storm event sampling



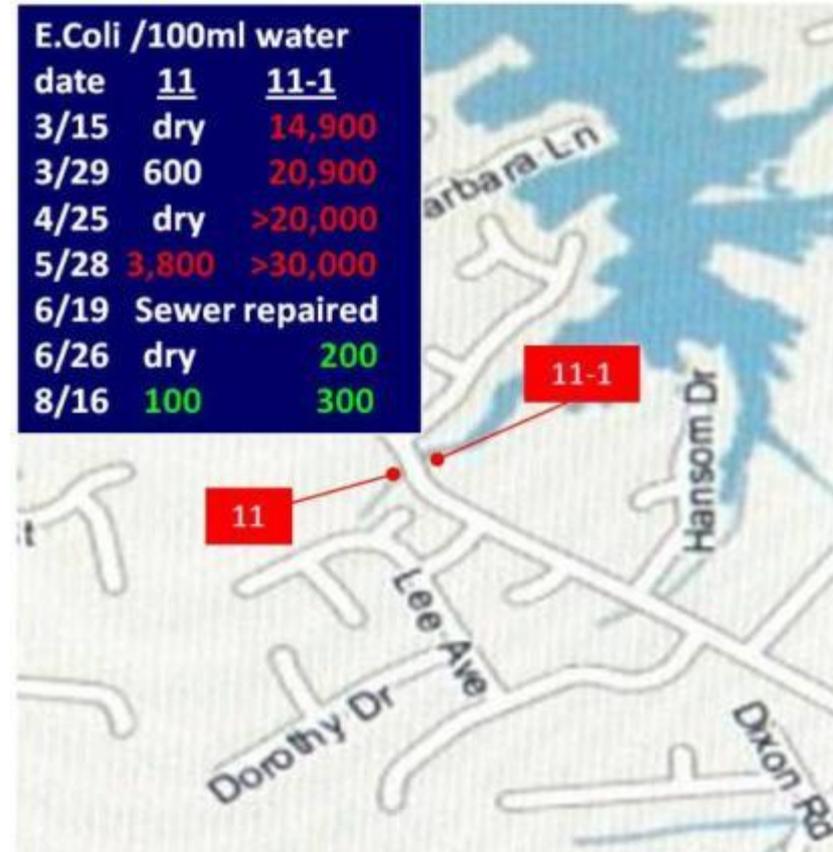
# SHOTGUN METHOD

- Set up sample stations scattered in the watershed
  - Sampling from public access points like bridges
- Ideal for initial recon to find E. coli ‘hotspot’ segments
  - Moderate labor and cost intensive



# TARGETED SAMPLING

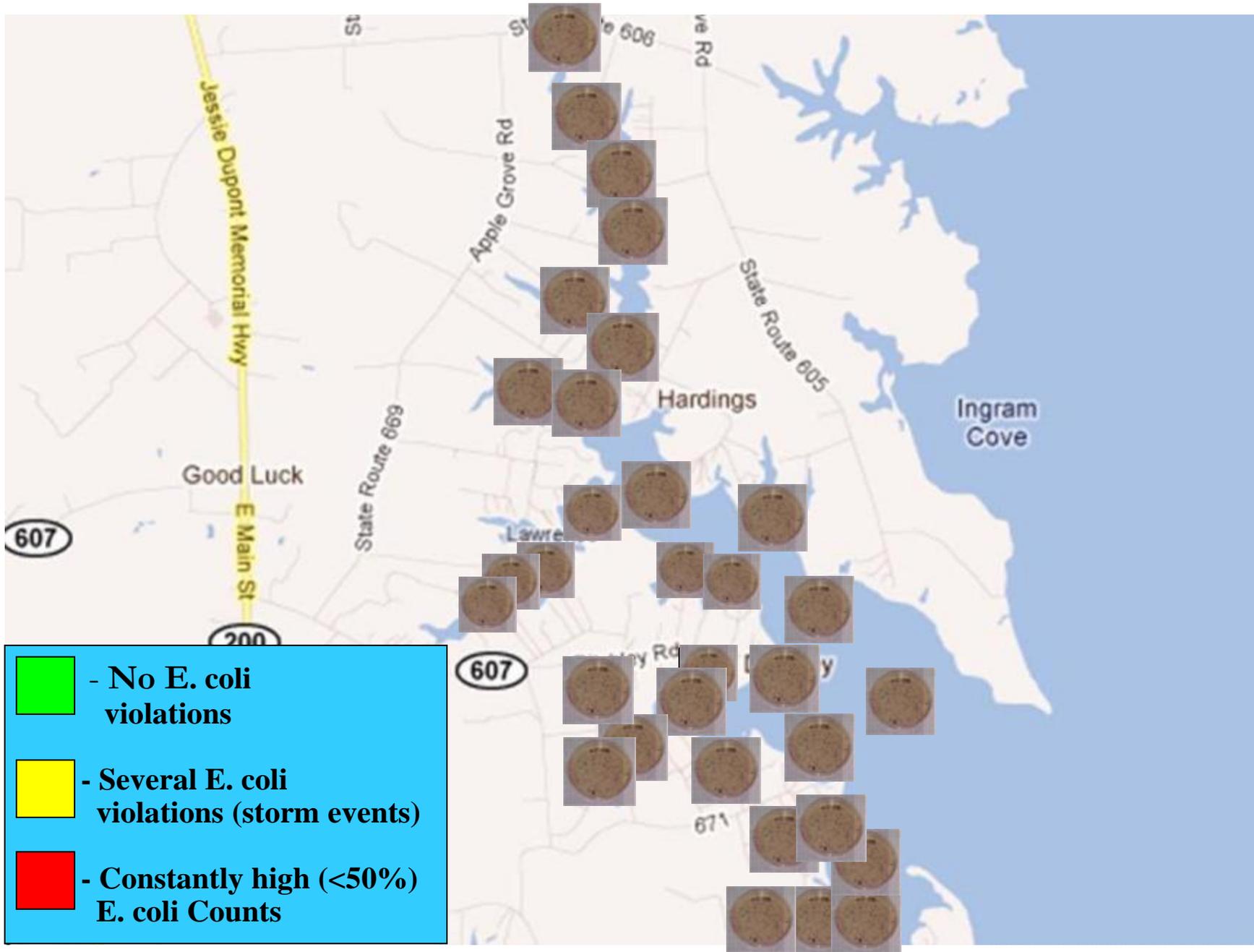
- Sample likely sources of fecal bacteria
- Sewer line crossings or near septic systems
- Areas of known dense wildlife or animal activity
  - Good to quickly rule out or confirm fecal sources
  - Requires planning to identify sources and may require landowner access



## CARPET SAMPLING

- Sampling every possible source and waterbody segment
- Extremely labor and cost intensive
- Provides the most detailed picture of fecal sources





## STORM EVENT SAMPLING

- Sampling at the first stages of a major rainstorm
  - Shows the worst case scenario of site bacteria levels
  - Helpful in identifying sources of bacteria not readily seen during dry sample events
- Most effective if samples are collected within the first hour of a storm
- $>1/4$ " rainfall is often enough to elevate bacteria levels
- Storm event sampling can be a part of any plan



# BEFORE HEADING OUT

- Let someone know you are heading out to sample and when you expect to be back
  - If possible, it is good to have a co-sampler join you
- Check the weather prior to going out
  - Dress appropriately for expected field conditions
- Inspect equipment
  - Needed sample equipment is in good condition (coolers, sample bottles, marker, sample bucket, etc.)
  - Have backup sample bottles if needed
  - Have safety equipment (first aid kit, flares, etc.)



## IF SAMPLING ALONG A ROAD

- If a safe parking area away from the road is not available, pull completely off the road and onto the shoulder
  - Park away from sharp curves
  - Leave sufficient room to exit/enter the vehicle
  - Turn on the vehicle hazard signal
  - If possible, wear a reflective or orange safety vest
  - Try to exit away from the path of motor vehicles



## BRIDGE SAFETY

- Be aware of traffic conditions
- If there is a no loitering or fishing sign, best not to sample on the bridge
- Sample on the upstream or the safest side of the bridge

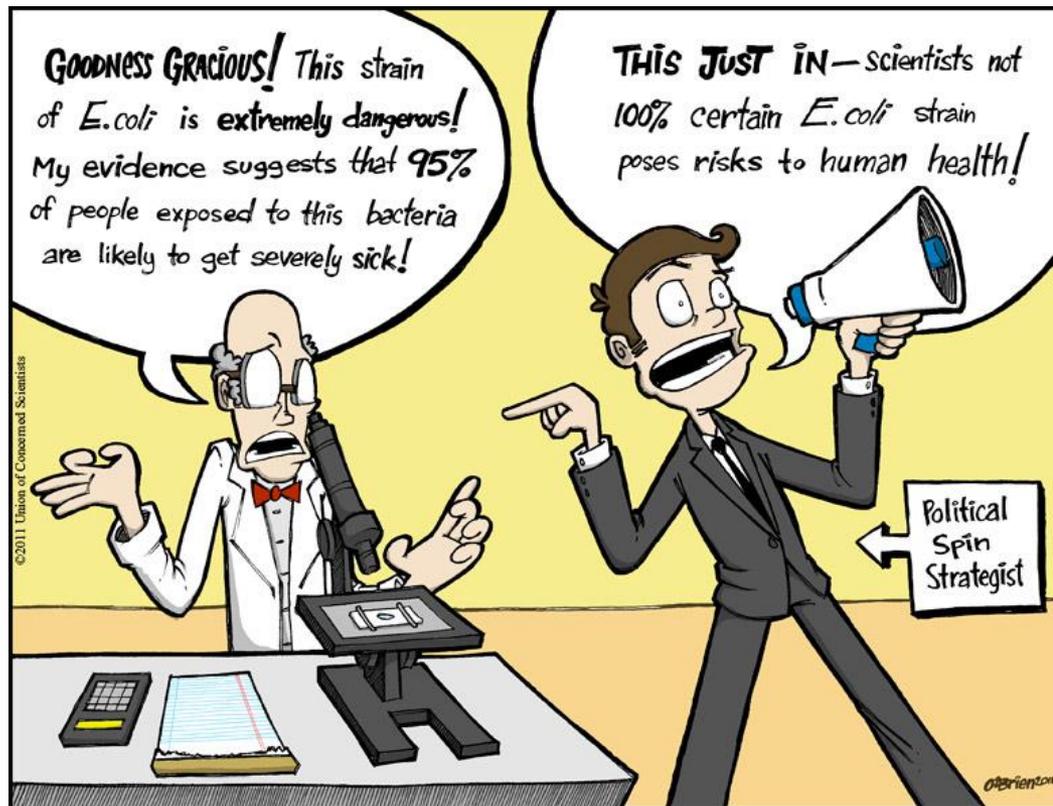


## CONTAMINATED WATER

- Use extra protection if water has an unusual odor or color
- Wear gloves or have hand sanitizer available during sampling
- Wash or disinfect hands after sampling and prior to eating
- Always assume sample water contains pathogens!



# QUESTIONS



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