Pathogen Indicators of Recreational Water Quality

Water Monitoring Coordinating Council
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Epidemic versus Endemic Disease

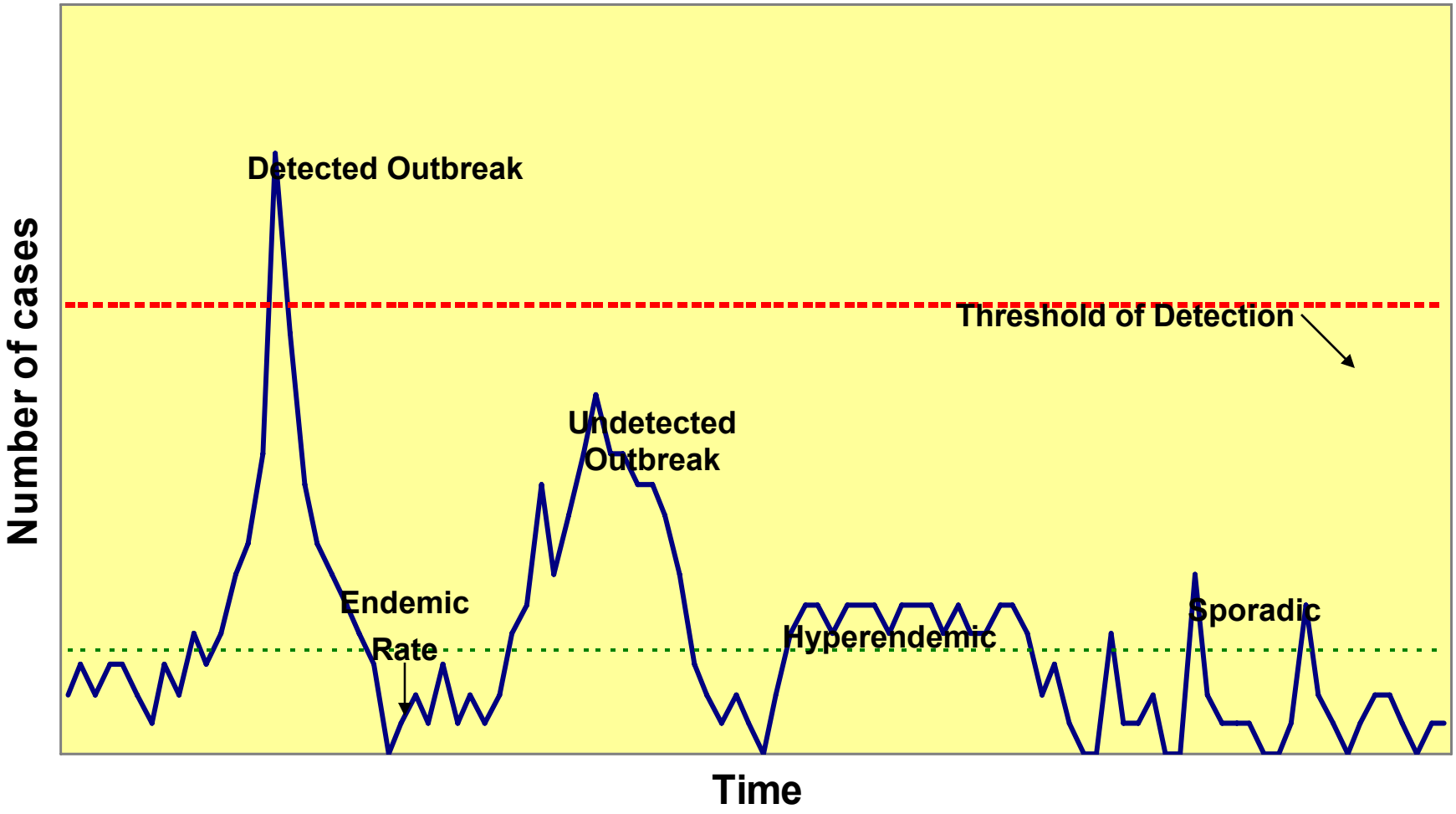


Figure courtesy of Rebecca Calderon, USEPA

CDC: Recreational Water Disease Outbreak Data

- Since 1998 the no. of outbreaks in treated waters (pools, spas, waterparks, fountains) has surpassed that in untreated waters (rivers, lakes, reservoirs).

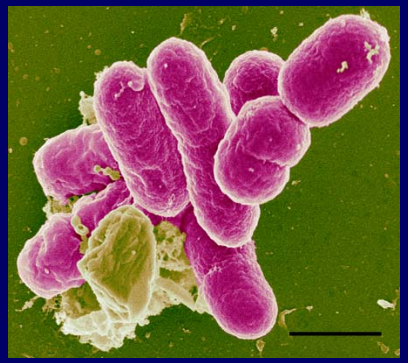
- Many outbreaks are due to immediate human-to-human transmission (e.g., from dirty diapers).

Note: It's not all about poop.

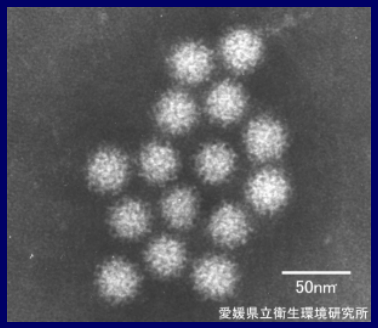
- Many but not all microbial pathogens in environmental waters are derived from fecal pollution.
- Poop is mostly microbes: most are good guys, some had deprived childhoods.

The Poop Groups:

- **Bacteria** (*E. coli*, Shigella, Vibrio).
  - Only group that can multiply outside of host.
  - 0.5 - 8 µm.
- **Viruses** (hepatitis A, rotavirus, Norovirus).
  - ~1200 genotypes (2-5x bacterial diversity).
  - Smallest size group: 0.025 - 0.09 µm.
  - Human sources almost exclusively.
- **Parasites** (Giardia, Cryptosporidium).
  - Some very resistant to chlorination.
  - 1-50 µm or more.



Shigella flexnerii



Norovirus

Giardia lamblia

Number of reported disease outbreaks and cases in untreated waters, by etiology, in U.S. 1997-2004			
Etiology	Outbreaks	Cases	
Bacteria			
Vibrios (monitored 03-04 only)		142	*
E. coli	9	136	
Shigella	5	96	
Plesiomonas	3	37	
Leptospira (urine)	4	401	
Pseudomonas	1	50	
Viruses			
Noroviruses	9	384	
Parasites			
Cryptosporidium	5	237	
Giardia	3	29	
Naegleria		17	**
Shistosomes	2	21	
Unknown	16	1156	
* 9 fatal.			
** All fatal.			
Yellow = fecal pollution-related.			

~390 cases/yr.

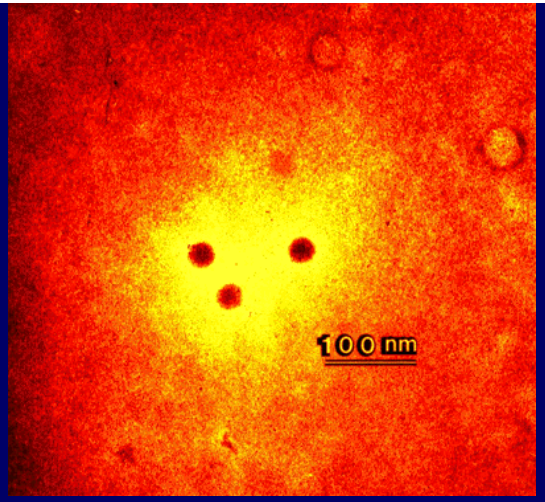
CDC Disease Estimates:

Salmonella typhi

Waterborne disease in the US:
900,000 illnesses and 900 deaths per year.

[Foodborne disease: 76 million illnesses and 5,000 deaths per year]

All are small.
Some are really small.



- Thickness of a paper clip = 1 mm (= 1,000 µm)
- Giardia cyst (length) = 14 µm 71 per paper clip width.
- A white blood cell = 12 µm 83 “
- Cryptosporidium oocyst = 5 µm 200 “
- E. coli (length) = 2 µm 500 “
- microsporidia spores = 1 µm 1000 “
- rotavirus / adenovirus = 0.07 µm 14286 “
- hepatitis A virus/Norovirus = 0.03 µm 33333 “
  - 64 million of these could fit inside an empty white blood cell

Intestinal Bacteria

(indicator groups in color)

E. coli – photo by Dennis Kunkel

- Bacilli (rods or barrel shaped)
  - Clostridia (C. coccoides & C. leptum subgrps.)
    - pathogens = C. perfringens, C. difficile
    - Eubacteria, Faecalibacterium, Fusobacterium.
  - Bacteroides
    - Prevotella
  - Bifidobacterium
  - Atopobium
    - Collinsella
  - Lactobacillus
  - Desulphovibrio
  - **Enteric bacilli** & similar bacilli (0.1% of molecular probed cell count in human feces)

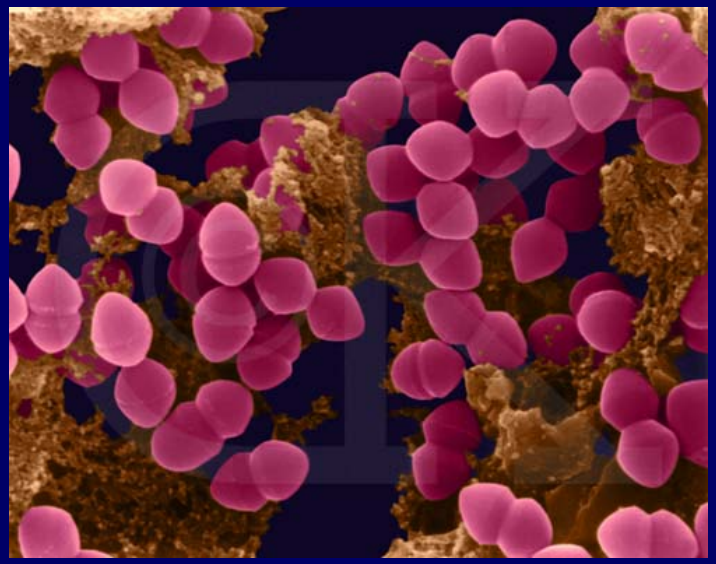
“Enteric” and Similar Bacilli

- **Enteric Bacilli**
  - Citrobacter
  - Edwardsiella
  - Enterobacter
  - Erwinia
  - Escherichia
  - Klebsiella
  - Plesiomonas
  - Proteus
  - Salmonella
  - Serratia
  - Shigella
  - Yersinia
  - Others

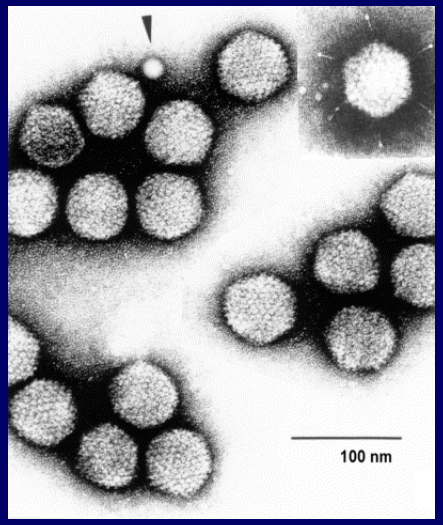
- **Similar Bacilli**
  (gamma-proteobacteria)
  - Vibrio (curved rods)
  - Aeromonas
  - Francisella
  - Pasteurella
  - Pseudomonas
  - Acinetobacter
  - Others

Intestinal Bacteria (con't)

- Cocci (round shaped bacteria)
  - Ruminococcus
  - Peptococcus, Peptostreptococcus
  - Streptococcus
  - **Enterococcus**
  - Neisseria, Veillonella
- Others
  - Spirochetes (spiral shaped; e.g., *Leptospira interrogans*).
  - Mycoplasma (no cell wall).
  - Archaean: Methanobrevibacter.
- ~25% still unknown.

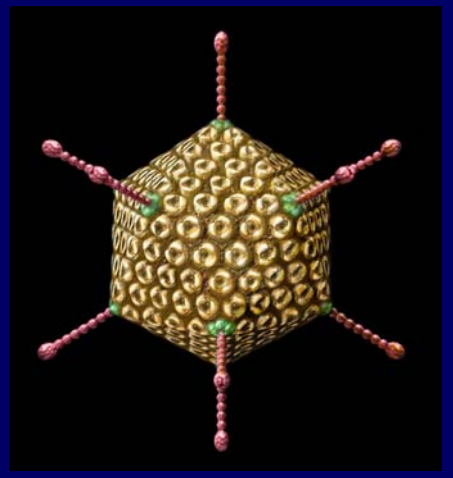


Enterococci. Photo by Dennis Kunkel

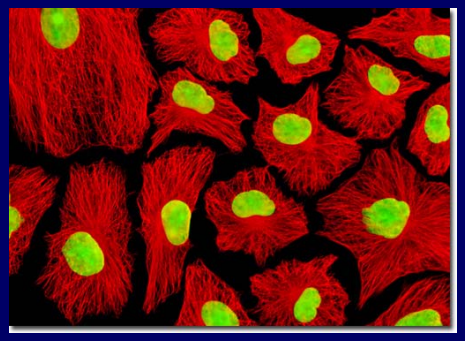


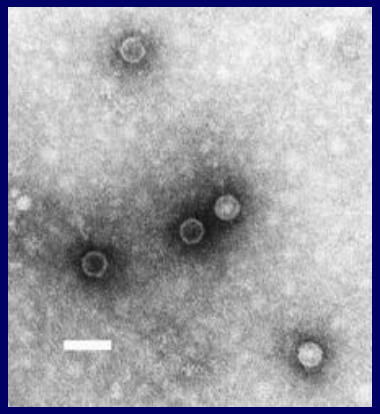
Adenovirus

Intestinal DNA Viruses


Adenovirus

- Adenovirus
  - 51 human types
  - very UV resistant
  - dsDNA
- some Parvovirus
  - ssDNA virus.

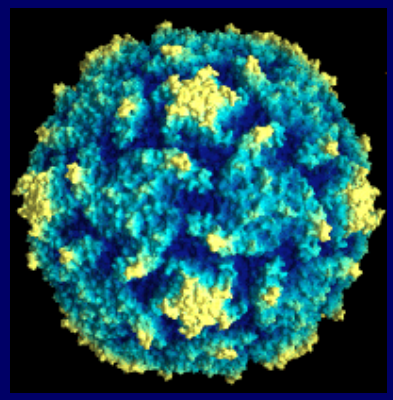




Poliovirus

Intestinal RNA Viruses

(color = major players)



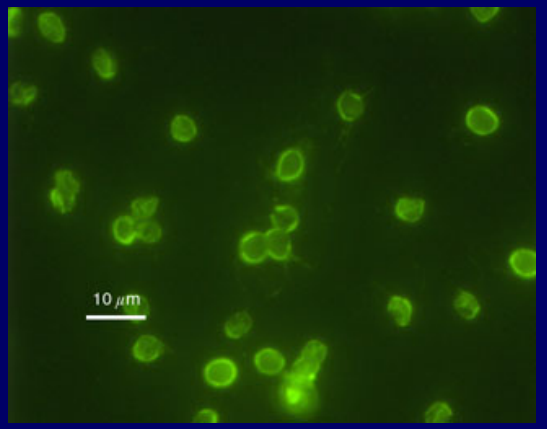
Poliovirus

- Astrovirus
- Calicivirus (Hepatitis E virus, **Norovirus**, Sapovirus).
  - Norovirus: a/k/a Norwalk-like or SRSV; est. 93% of non-bacti gastro outbreaks in US; 4 groups–hundreds of strains;)
- Coronavirus (pathogenic? waterborne?)
- Flavivirus: Pestivirus (waterborne?)
- Picobirnavirus (waterborne?)
- **Picornavirus (cultivable group)**
  - Enterovirus (types 71 & 68), Coxsackievirus (types A9, B1, B5), Echovirus (types 9, 30, 7), Poliovirus (vaccine strain only in US), and Hepatitis A virus (vaccine available).
- Reovirus (Reovirus, **Rotavirus** [humans: 6 Group A serotypes mostly; vaccines available]) (cultivable also).

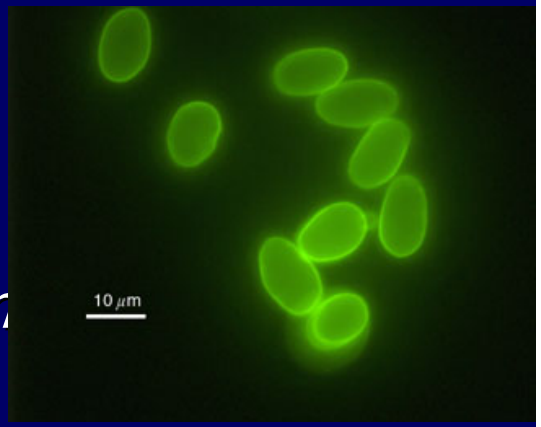
Parasites

(color = major players)

- Protozoa (single cell; eukaryotes)
  - *Balantidium coli*
  - ***Cryptosporidium***
    - *C. parvum and C. hominis.*
  - *Entamoeba histolytica*
  - ***Giardia lamblia (G. doudenalis)***
  - *Cyclospora cayetanensis (mostly foodborn*
  - microsporidia
  - *Naegleria flowleri*.
- Other: *e.g.*, Schistosomes (e.g., Trichobilharzia),

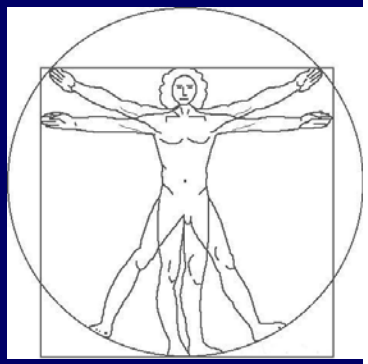


Cryptosporidium parvum oocysts



Giardia lamblia cysts

Everybody poops.



- Human pathogens of concern: all groups
- Animal pathogens of concern: bacteria, protozoa (NOT viruses).
- Animal sources include wild as well as domestic animals.
  - Pathogens in a watershed can be reduced, but not eliminated.

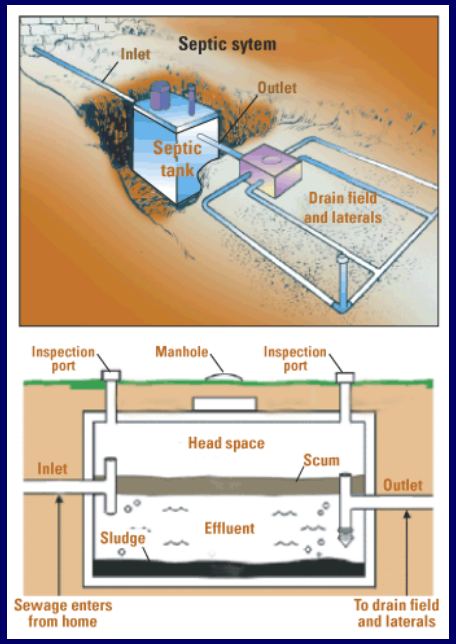
Whose poop is worse?

- Generally speaking, the order of risk of infection to humans is: human > domestic animal > wild animal feces.
- Many pathogens host-specific: (*e.g.*, Salmonella typhi, Shigella, Vibrio cholerae, Entamoeba, many viruses).
- Many more recorded disease outbreaks due to domestic animals than indigenous animals.
- But, the amount of difference in risk is unknown.
  - *e.g.*, birds are carriers of Salmonella, Campylobacter, and other human pathogens.

Goose feces

Where do YOU poop? Where does it go?

- Treated or partially treated human sources:
  - Septic tank effluents.
  - Sewage treatment plant effluents.
  - Land-applied STP-treated sludge (Federal 503 regs. control this practice).
  - Septage disposal.



Relative Resistance of Microbes to Chlorine Disinfection[a]

- E. coli 1X
- Poliovirus 36-50X
- Giardia lamblia <1,000-7,600X
- Cryptosporidium parvum 686,000X

- [a] C.R. Sterling, p.58 in Dubey, J.P. *et al*, eds. 1990. *Cryptosporidiosis of Man and Animals*. CRC Press.

Indicator Concentrations at a Large Wastewater Treatment Plant [a]

Sample	Date	E. coli (cfu/100 ml)	Ent (cfu/100 ml)	CP (cfu/100 ml)	Som Phage (pfu/100 ml)	F+ Phage (pfu/100 ml)	Coprostanol (µg/L)
Untreated	09/25/01	4,000,000	975,000	78,000		47,000	744
	01/15/02	7,000,000	4,200,000			120,000	2136
Treated, before chlorination	06/14/00	200,000	3,200	2,360	>2,000	2,100	23
	09/25/01	740,000	75,000	2,100		500	6
	01/15/02	720,000	250,000			20,320	690
Treated, after chlorination	06/14/00	64	0	3,600	1,750	2,000	11
	09/25/01	3	0	1,560		200	4
	01/15/02	2	1			16,020	747

[a] Primary clarification, aeration with recycled sludge, secondary clarification, chlorination. Capacity: 40 million gallons per day (151 million liters per day).

Ent = enterococci; CP = Clostridium prefringens; cfu = colony forming units.

Som phage = Somatic coliphage; F+ Phage = F+ coliphage; pfu = plaque forming units.

ug/L = micrograms per liter.

Not done.

Where do YOU poop (con't)

- Untreated human sources:
  - Malfunctioning or overloaded STPs and septics (esp. during heavy rainfall?).
  - Sewage collection pipe breaks (important for ground water).
  - Combined storm/sanitary sewers (mostly a lower Delaware R. and NY/NJ Harbor issue).
  - Storm drains (illegal sanitary connections, cities: waste from homeless people).
  - Boat discharges.
  - Landfill leachates (disposable diapers).

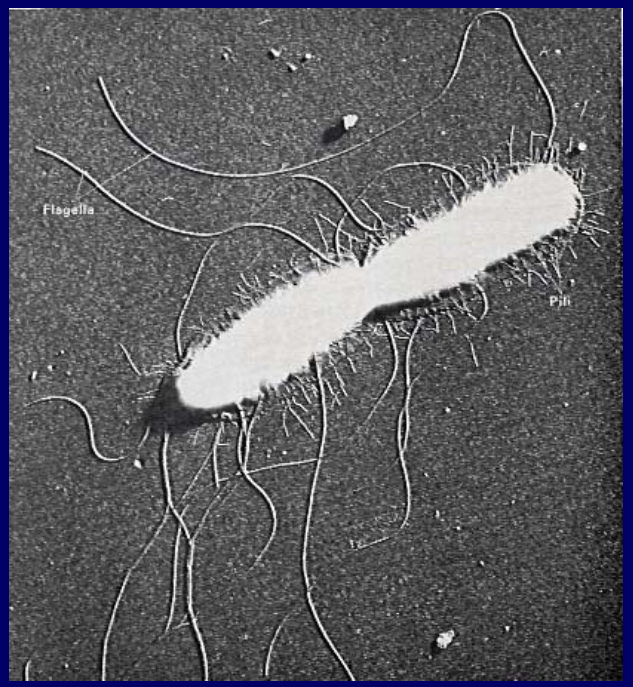
Does a bear poop in the woods?

- Treated or partially treated animal sources:
  - ??
- Untreated animal sources:
  - Storm drains (waste from birds, dogs, rodents etc.; rodent habitat).
  - Storm water runoff (farms, feedlots, all land).
  - Direct inputs (rodents, waterfowl, cattle grazing in streams).
  - Landfill leachates (some pet wastes).

How come we're not all nose deep in poop?

- Pathogens die off once they enter the environment.
  - In sediments and soils in warm conditions, bacteria (not viruses or protozoa) can multiply.
- Rate of die-off is pathogen-dependent:
  - Some die off very quickly (Shigella), some take months or years depending on conditions (some viruses and parasites).
- From a pathogen's point of view:
  - Sunlight = bad. Darkness = good.
  - Warm temperature = good or bad. Cold temperature = good.
  - Particulate matter = good.
  - Nutrients = good (for bacteria).
  - Other microbes = very bad.

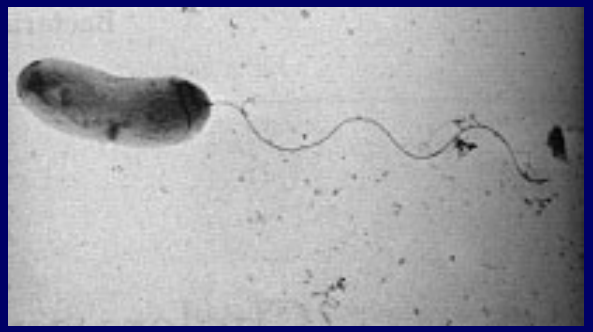
A microbe in the water column is temporarily misplaced.



S. typhi, 15,000x, from T. Brock, *Biology of Microorganisms*.

Microbes are “particles”. They prefer attachment to particles, surfaces, each other, or to infect a host.

Do we look for pathogens?



Vibrio cholerae

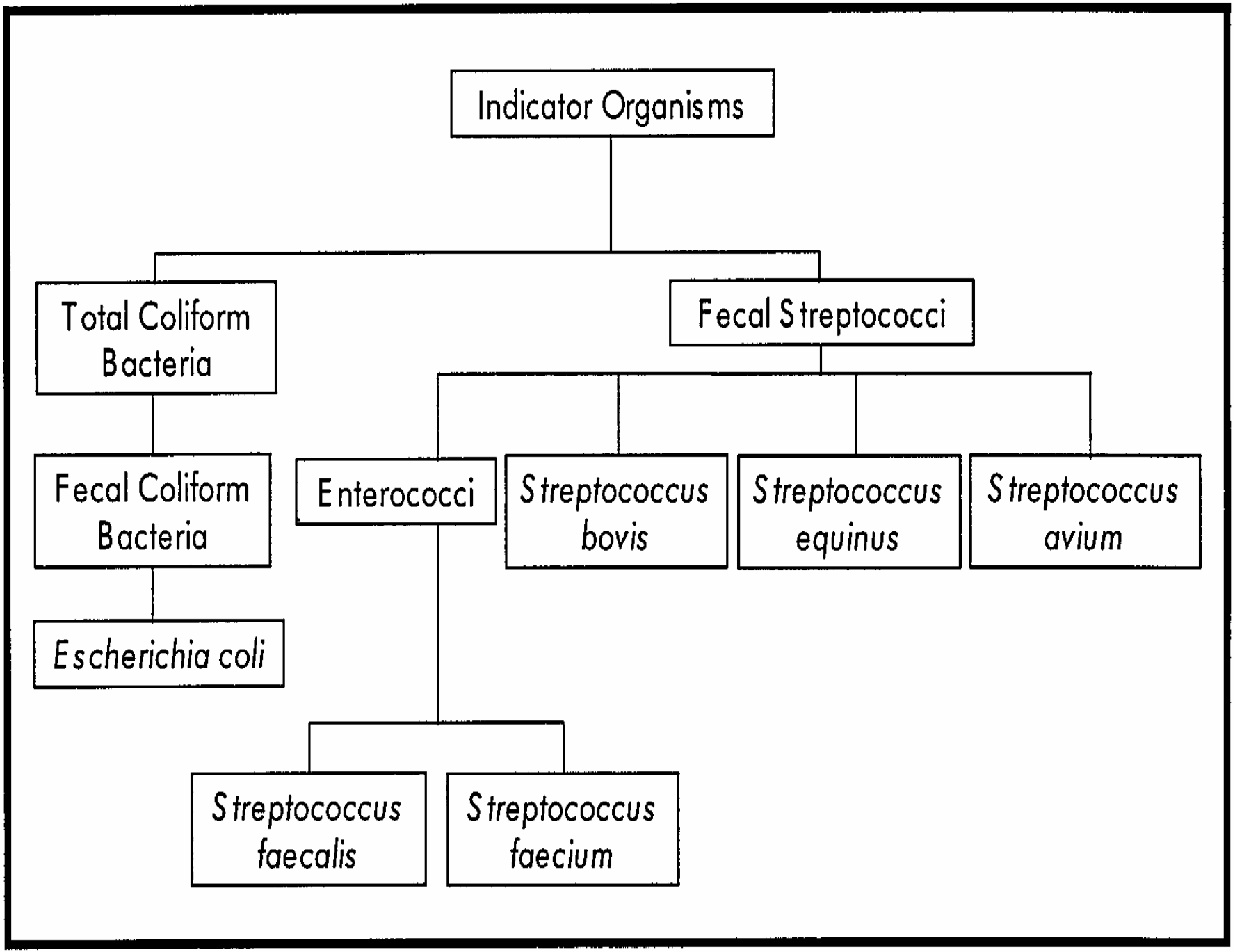
- No.
- Not always present in fecal wastes.
- Different pathogens are present at different times.
- May be present in low numbers.
- Infectious dose is different for each pathogen.
- Many difficult or impossible to grow in the lab.
- Many tests difficult, expensive, time-consuming.

If it looks like poop, it must be poop.

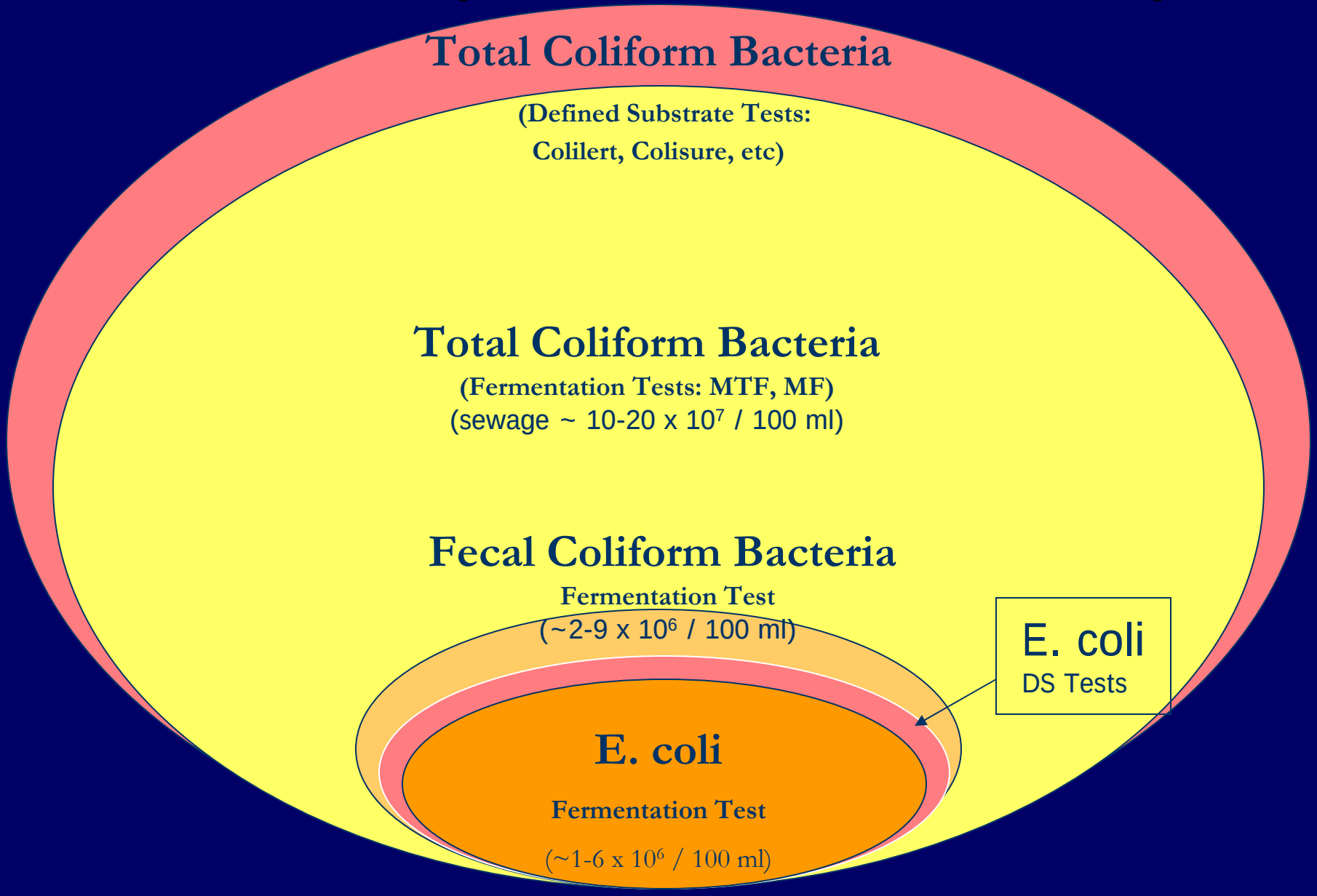
- We measure “indicators” of fecal pollution.
- Total Coliforms, Fecal Coliforms, *E. coli*.
- Enterococci, coliphage (GWR).
- Good for most, not all bacterial pathogens.
- Currently, no specific indicator of viral or protozoan pathogens.
  - Possible candidates: coliphage, *C. perfringens*, others?

Characteristics of "The Perfect" Fecal Pollution Indicator

- Always present in fecal wastes.
- Only found in fecal wastes.
- Found in greater numbers than any pathogen.
- Should not be able to multiply in environment.
- Should have transport and survival characteristics similar to pathogens.
- For water treatment processes: should react to filtration & disinfection as do pathogens.
- Detection method should be fast, easy and inexpensive for routine use.

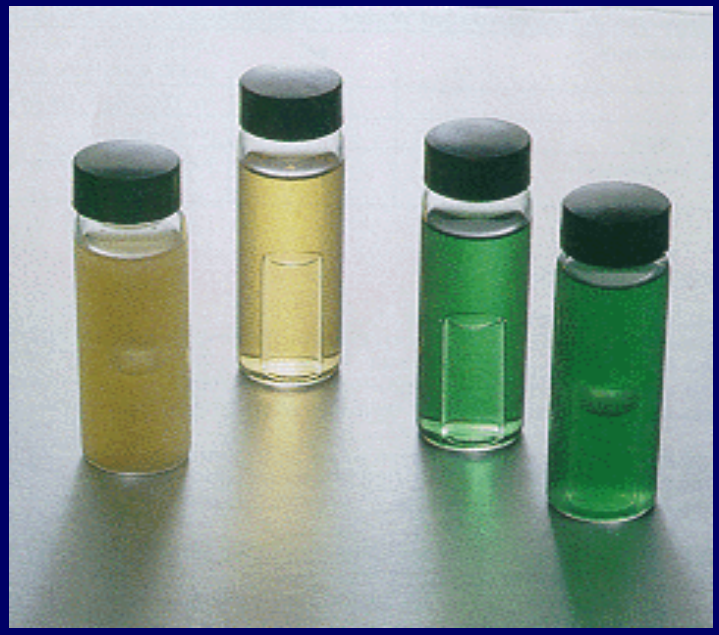

Indicator Organisms
Total Coliform Bacteria
Fecal Streptococci
Fecal Coliform Bacteria
Enterococci
Streptococcus bovis
Streptococcus equinus
Streptococcus avium
Escherichia coli
Streptococcus faecalis
Streptococcus faecium

Relationship of Coliform Groups

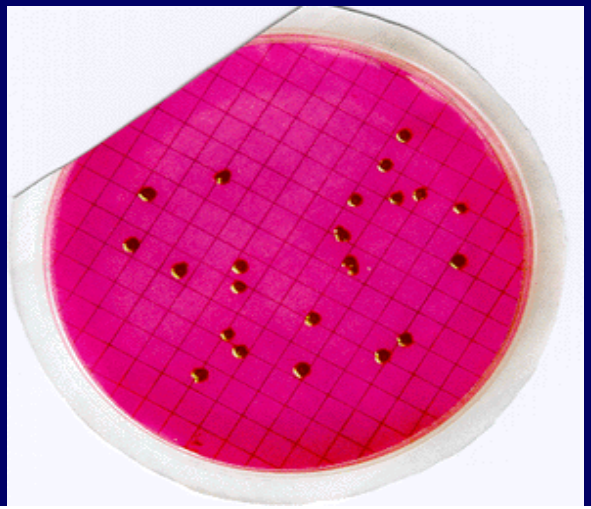


Total Coliform bacteria:
Fermentation Tests

- MPN:
  - Presumptive (Lauryl tryptose broth - growth, acid or gas) & confirmed (BGLBB broth - gas) tests.
  - 48 +/- 3 Hrs; 35 +/- 0.5 ^{o}C.



- MF:
  - Red colonies with metallic sheen on Endo agar.
  -



Total Coliform bacteria:
Defined Substrate (enzyme) Tests

- Colilert®, Colisure®, others.
  - Coliform enzyme (β-galactosidase) converts a colorless "lactose surrogate" (e.g., "ONPG") to a colored product.
  - Type of color depends on which test used.
  - These tests determine *E. coli* at the same time.

Total Coliform bacteria

(major component in yellow)

- Escherichia (not all strains)(F)
- Enterobacter (F)
- Klebsiella (F)
- Citrobacter(F)
- Serratia (F)
- Leclercia (F)
- Yersinia (F)
- Others (Hafnia, Buttiauxella, Kluyvera, Pantoea, Rahnella)(F)

F = **may** be free-living (may not be fecal-derived)

Fecal Coliform bacteria:

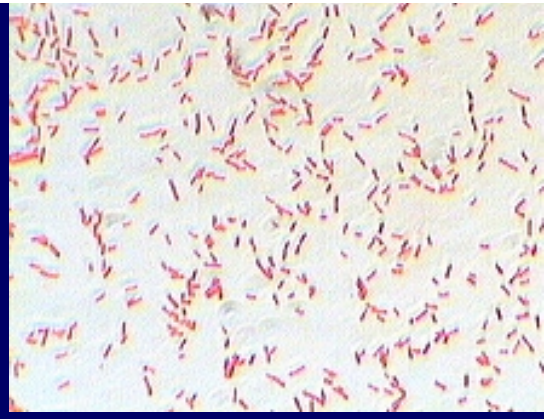
Fermentation Test

- Gas production in EC medium after 24 +/- 2 h at 44.5 +/- 0.2 ^{o}C.

- Fecal Coliform Bacteria:
  - Escherichia (not all strains)(F)
  - Klebsiella (F)(15% of *K. pneumoniae* strains)
  - Enterobacter (F)(5% of *E. cloacae* and *E. aerogenes* strains)

F = **may** be free-living (not fecal-derived)

Escherichia coli (E. coli)

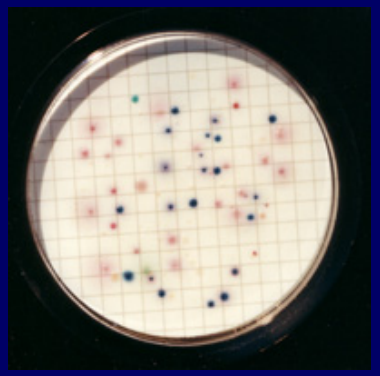


- Named for Theodor Escherich.
  - First to observe“Bacillus coli”, “Bacterium coli”.
- Historically, thought not pathogenic.
  - Many pathogenic strains now known, some serious (*e.g.*, E. coli O157:H7).
- Historically, thought to be fecal-specific.
  - Free-living populations now apparent in both tropical and temperate climates.
- Easy assays developed in 1980’s.
  - based on presence of ß-glucuronidase.

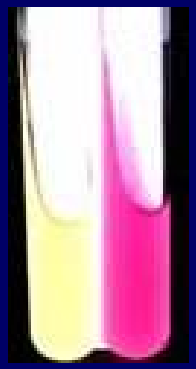
E. coli

Colilert-Quantitray

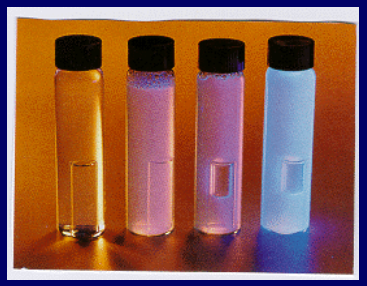
Colilert-P/A



Coliform/E. coli MF test



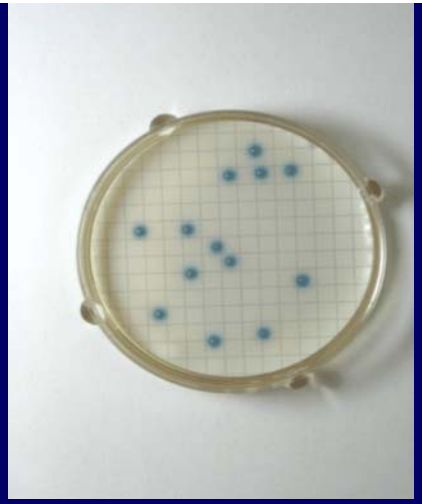
Urease test



- Fermentation Test:
  - Membrane filter test.
  - mTEC medium + urease test.
- Defined Substrate Tests:
  - Colilert®, Colisure®, E*Colite®, M-Coliblue 24®, Chromocult®, Readycult®, EC medium+“MUG”, nutrient agar+“MUG”; 35 ºC.
  - E. coli has ß-glucuronidase enzyme.
    - Converts 4-methylumbelliferyl-ß-D-glucuronide (**“MUG”**) (colorless) to 4-methylumbelliferone (fluorescent under UV light).

Enterococci

(formerly group D Streptococci)

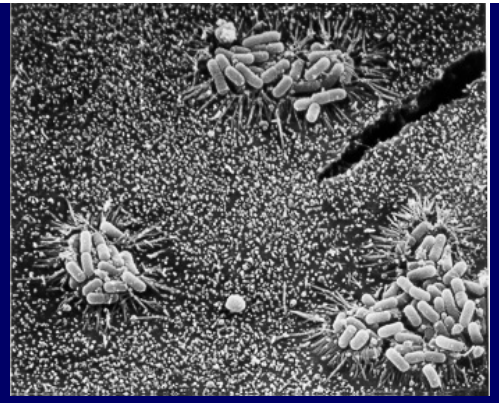


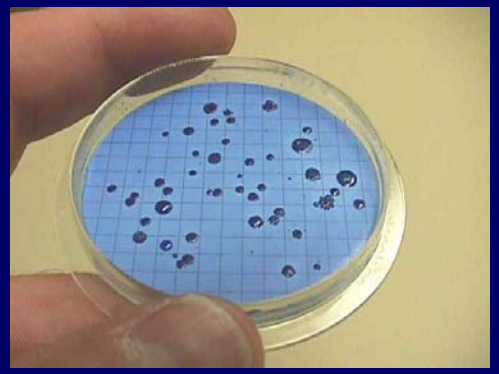
- Growth on mEI medium, 24 h, 41°C to produce colonies with a blue halo.
  - mEI =peptone, yeast extract + esculin, NaCl, actidione, sodium azide.
- 19 Species of Enterococcus + *S. bovis, S. equinis*.
  - e.g., *E. faecalis, E. faecium.*
- Some free-living members also.
- Method 1600. Defined substrate test.
  - Enterococci have α-glucosidase enzyme.
  - Converts indoxyl-α-D-glucoside (colorless) to indigo (blue).

Meaning of Positive Indicator Tests

Test	Does a positive test indicate fecal contamination? [a]	Notes
Total coliform	suggestive	High concentrations in intestinal wastes but non-intestinal sources also.
Fecal coliform	likely	Minor non-intestinal component in many but not all waters.
E. coli	likely	Minor non-intestinal component in many but not all waters.
Enterococcus	likely	Minor non-intestinal component in many but not all waters.
[a] Suggestive = confirmatory testing necessary. Likely = more definitive than TC test but may contain non-intestinal sources.		

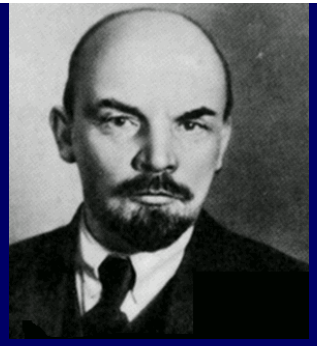
Microbial Nomenclature





- **Quantitative Example:**
  Total Coliform = 35 MPN/100 ml or 35 CFU / 100 ml.
- **Per 100 ml** = Convenient volume. Milk sample bottles used in sanitation labs years ago [3.4 ounces or 0.2 pint].
- **MPN** = **m**ost **p**robable **n**umber, from multiple test tube growth or fermentation (MTF) assays.
- **CFU** = **c**olony **f**orming **u**nit (membrane filtration - MF).
- MPN = CFU ≅ single organism.

Pre-1976 Pathogen Criteria



1924: Lenin dies

1948: Truman defeats Dewey

- Were region-specific and generally what could be attained, not what was safe.
- Based on observational information or Salmonella detection frequencies and not bather illness.
- 1924: USPHS Committee on bathing practices recommended total coliform monitoring for pools.
- Total Coliform (TC) Limits (per 100 ml):
  - Tennessee Valley (1945): 50
  - New York City (1948): 2,400
  - Most others (Potomac River, Great Lakes, Upper Mississippi, Ohio River & Connecticut-TC vs. Sal. detect relationship): 500-1,000
  - Some are max. values, others are “averages” (but mean or geometric mean not specified).
  - Occasional exceedences allowed (20% in one case).
- Cal. WQ criteria 1952 & 1963.

N.T.A.C. Report – 1968*: Recommendations

1968: Nixon elected President

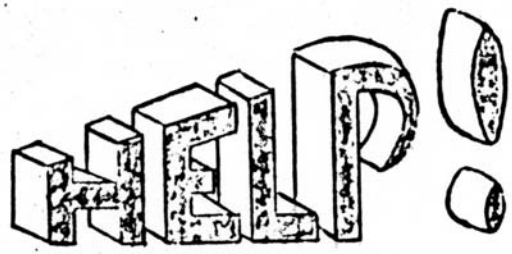
- 3 criteria recommended for:
  - 1) General rec. use of water [FC ≤ 2000 per 100 ml & ≤ 10% ≤ 4000];
  - 2) Enhanced rec. value of waters for rec. uses other than primary contact [FC ≤ 1000]; and
  - 3) Primary contact recreation.
- Primary contact recreation: Gm of fecal coliforms ≤ 200 organisms/100 ml based on ≥ 5 samples equally spaced within 30 days + no more than 10% of samples to exceed 400/100 ml.
  - pH 6.5-8.3; temp ≤ 80 oF. Potable and agric. water stds. also.
  - "Fecal strep. should not be used....not all FS revealed by the test and FS found in other sources such as plants and insects."
- Became the basis for the 1976 EPA Water Quality Criteria for Bacteria.

* *Fed. Wat. Poll. Control Admin. 1968. Water Quality Criteria. Report of the National Technical Advisory Committee to the Secretary of the Interior. Wash, DC, April 1, 1968.*

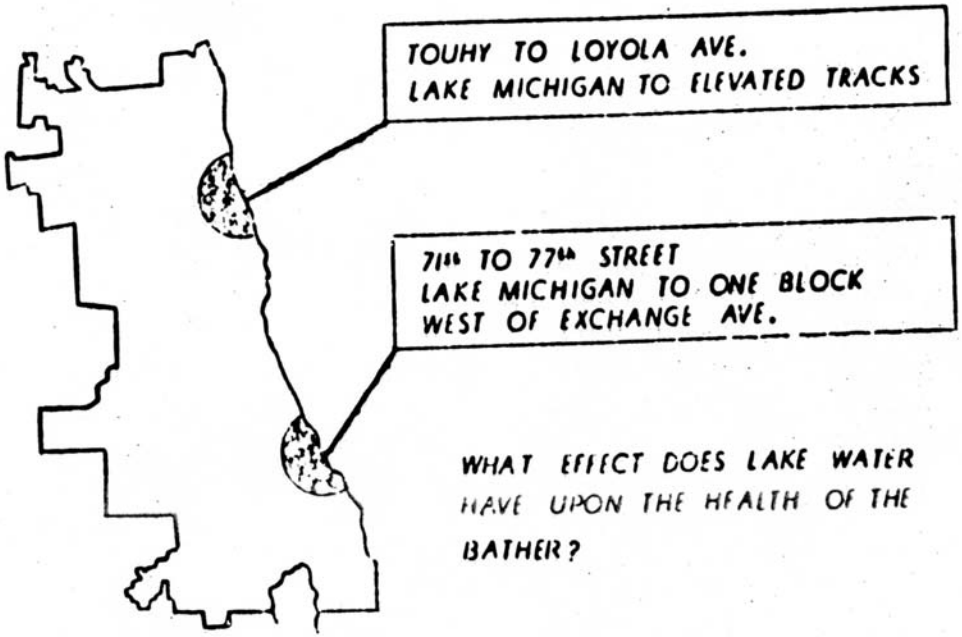
Basis of NTAC Recommendation (1976 EPA WQ Criteria)

From USPHS Epi. studies.

- Lake Michigan, Chicago, 1948.
  - "North beach"-Loyola Park area (Morse Ave; TC median=99 [9-3500]) & "South beach"-Rainbow Park area (75th St.; TC=176 [23-24,000]); no sewage enters Lake Michigan at Chicago.
  - 3-4 sample sites per beach area; 4-5 times/week; 2-foot depth; 6" below surface; SM9221B; 7/8-8/26.
- Ohio River, Dayton, KY, 1949.
  - "Dayton Bar" 1000 ft-long sand-gravel bar (TC Gm = 2,700 [230-160,000]) and Tacoma Park chlor. swimming pool (TC=<3 [<2-22]); untreated sewage in river. Daily sampling (Beach=3 sites; Pool=2 sites).
  - Study period (6/27-7/31) shortened due to polio outbreak in mid July.
- Long Island Sound, 1950s.
  - New Rochelle-Hudson Park?? and Mamaroneck-Harbor Island Park.
- Local residents only; kept daily swimming & illness calendars.



...1000 FAMILIES FROM THE NORTH AND SOUTH SIDE AREAS NEEDED IN LAKE MICHIGAN BATHING WATER SURVEY



WHAT EFFECT DOES LAKE WATER HAVE UPON THE HEALTH OF THE BATHER?

FOR THE FIRST TIME IN MEDICAL HISTORY A COMPREHENSIVE SURVEY TO DETERMINE WHETHER OR NOT CERTAIN ILLNESSES OR IRRITATIONS MIGHT BE CAUSED BY BATHING IS BEING UNDERTAKEN. YOUR COOPERATION WILL HELP NATIONAL, STATE AND LOCAL HEALTH AGENCIES IN THEIR ATTEMPT TO FIND AN ANSWER TO THIS PROBLEM.

A NURSE OR MEDICAL STUDENT WILL CALL AT YOUR HOME WITHIN THE NEXT FEW DAYS TO EXPLAIN THE SURVEY. THIS PROJECT DESERVES YOUR SUPPORT.

• • • • • • • • •

(Sponsored by: local governmental health agencies in cooperation with Illinois, Indiana and Wisconsin State Health Departments.)

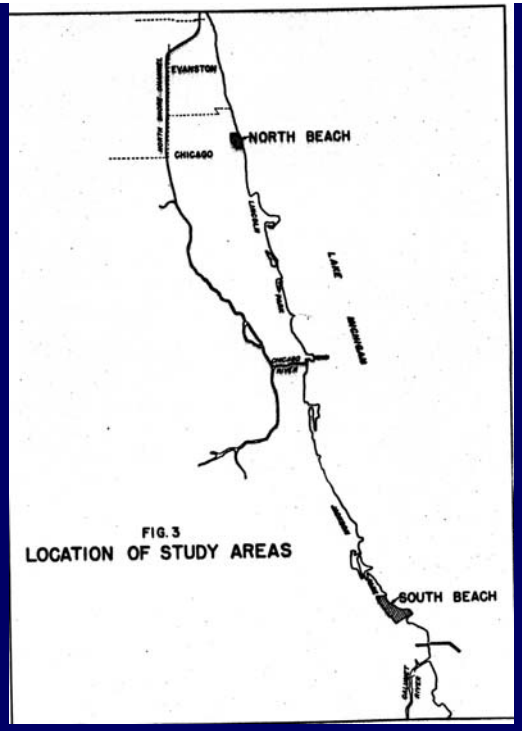
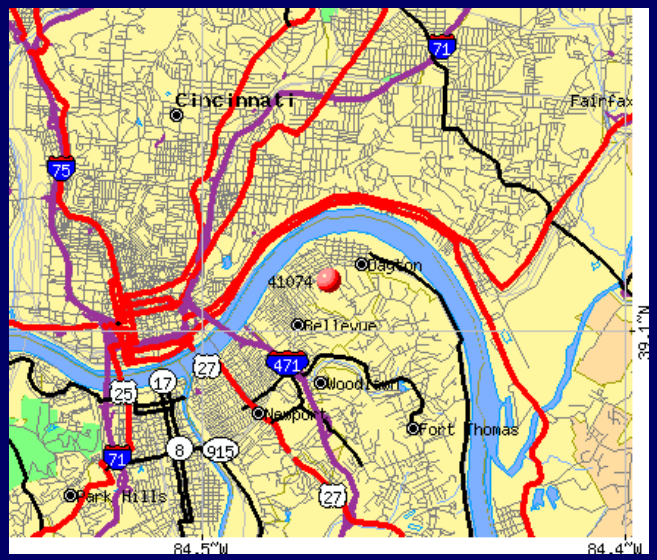
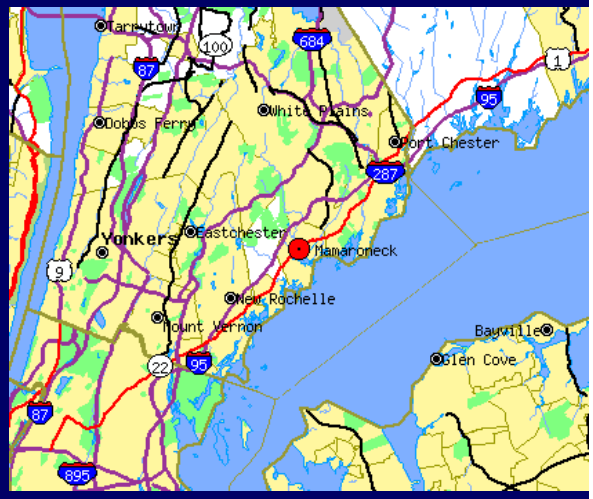


FIG. 3
LOCATION OF STUDY AREAS



Dayton, KY

(fresh)



Mamaroneck, NY

(marine)

Lake Michigan, Chicago 1925

(fresh)

Dayton Bar, Dayton, KY

New Rochelle, NY

present day

(marine)

EPA 1976 Recreational W.Q. Criteria

(a *de facto* freshwater criterion)

1976: Jimmy Carter elected President

- Significant increase in all illnesses, GI (~20%) and non-GI (~80%) at Lake Michigan when total coliforms at and above 2,300 per 100 ml (*).
- Fecal coliforms ~ 18% of total coliforms.
- 2,300 x 0.18 ≅ 400 (disease observed at 400).
  - Low virus levels at this concentration (**)
- 50% of 400 = 200.
  - Elevated Salmonella detection's above this concentration (***).
- Based on detectable rather than acceptable risk.
- Source of "10%" part of criterion - ??
  - Between 10-19 samples, no more than 1 sample > 400, so somewhat "analogous" to a single sample limit number for a bathing season with weekly sampling.
  - Disease observed at 400.

* During 3 "high coliform" days at Lake Michigan; South Beach (TC Gm = 2328) but not North beach (TC=732). Also, slight excess GI illness at the Ohio River beach (O/E ratio = 1.32; p=0.05). No swimming-related illness observed at NY beaches.

** In treated (non-disinfect.) sewage: When FC = 400, virus pfu = 0.02.

*** When FC ✂ 200, Sal detections ✂ 28%. When FC 201-2000, Sal detects 85-98% in freshwaters.

Shellfish Harvest Water Stds.

Regulated by FDA – NSSP, not EPA

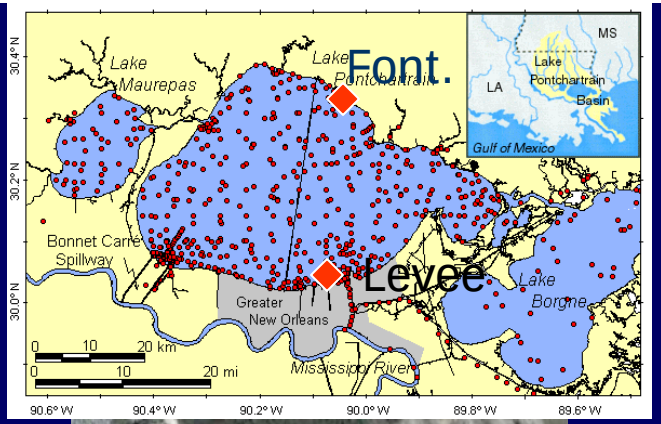
- Acceptable harvest waters, based ≥ 15 samples:
- Total Coliforms: median or Gm ≤ 70 per 100 ml & not more than 10% above 230 per 100 ml. OR
- Fecal coliforms: median of Gm < 14 per 100 ml and not more than 10% above 43 per 100 ml.
- Based on 1914-1925 state and PHS studies showing…

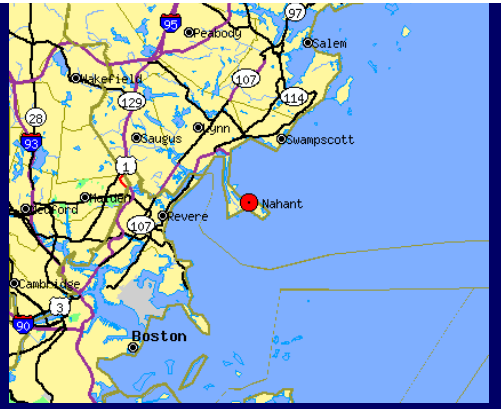
  "typhoid fever or other enteric disease not attributable to shellfish harvested from [TC ≤ 70 per 100 ml] waters. This count = fecal material from one person diluted in 8 million cubic feet of water. This small amt. of sewage reaching a growing area would be so treated, diluted or aged as to be of negligible public health significance."
- When TC ✂ 70, Salmonella detections ✂ 6.7%.

EPA 1986 Criteria: Marine Waters

1986: Challenger explodes

- Enterococcus Gm of 35/100 ml AND
- Single Sample limit (SSL) of 104-500/100 ml (75%-95% upper confidence interval limits); depending on designated use.
- 1972 CWA 304(a)(1) mandated new criteria.
- Epi. Studies at Coney Island, NY (73-75), Rockaway Beach, NY (73, 74), Lake Pontchartrain, LA (brackish; 77, 78), Revere & Nahant, MA (78).
- Studied 11 indicators/indicator groups.





Coney Island

(18-22nd St, then 4 separate beaches)

Fontainebleau S.P.

Revere, Mass

Rockaway Beach

(67th St, then Riis Park)

Levee “Beach”-Bayou St. John

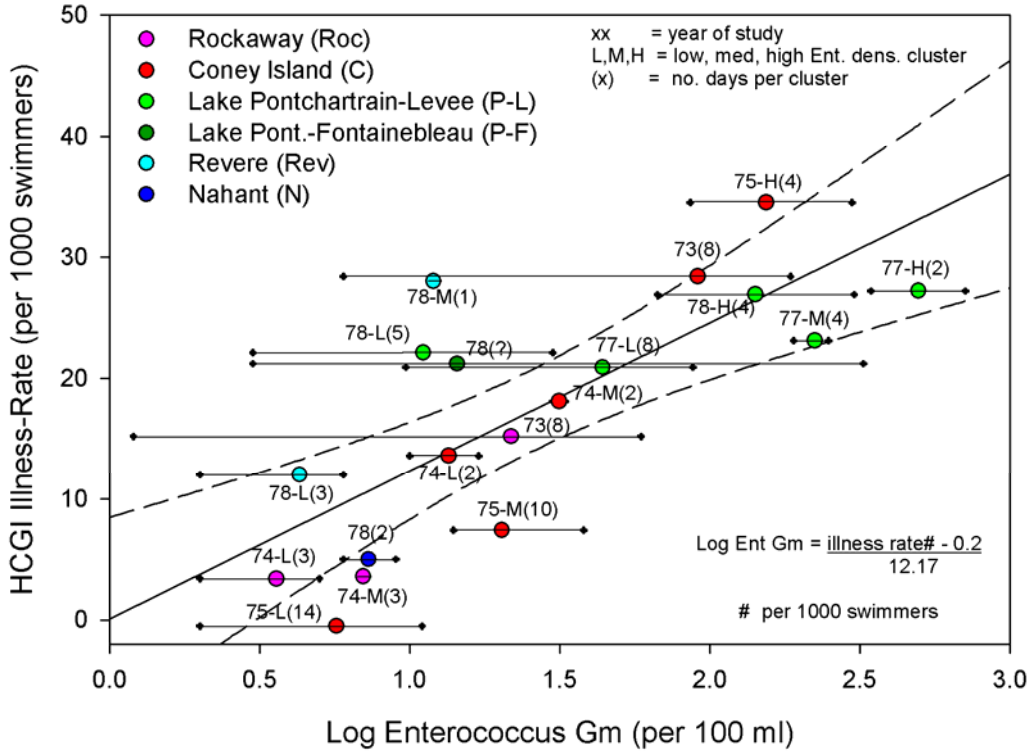
(Bayou mouth + roped-off area)

Nahant, Mass

Highly Credible Gastrointestinal Illness

- Vomiting OR
- Diarrhea & fever, or illness disabling enough to remain at home, or in bed, or seek medical advice OR
- Stomachache & fever or nausea & fever.

Enterococcus Density vs. Highly Credible Gastrointestinal (HCGI) Illness Rate

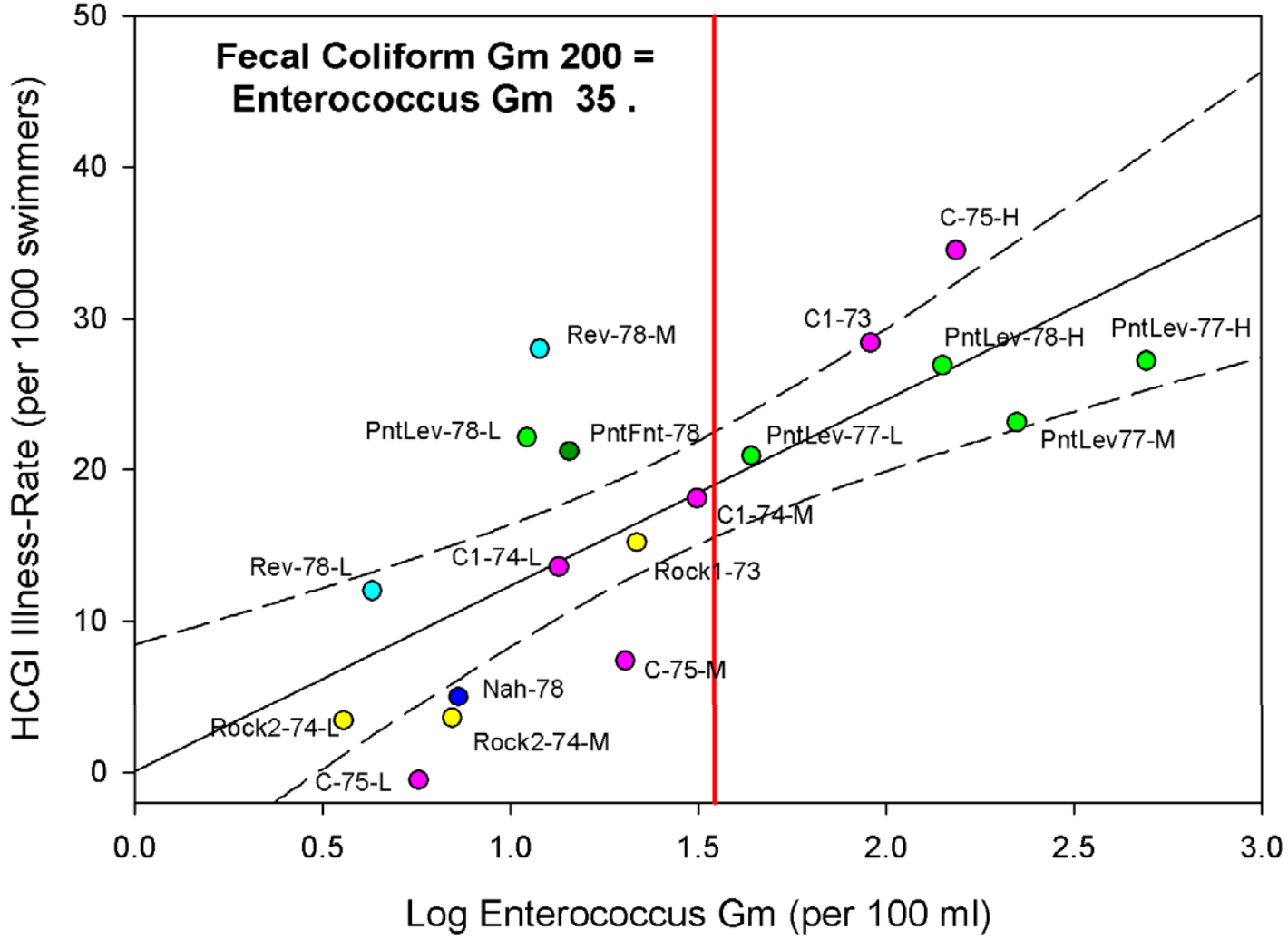


- "HCGI Illness rate" = Swimmer rate – Non-swimmer rate; 8-10 days after swimming. Linear regression model.
- Each data point = A group of "bathing days," per year, that had similar indicator densities ("clustered" data). Indicator Gm was calculated for that group of days (6-12 samples per beach per day).
- Data also analyzed per beach, per year.
  - Lower corr. coeff. than by clustering the data.

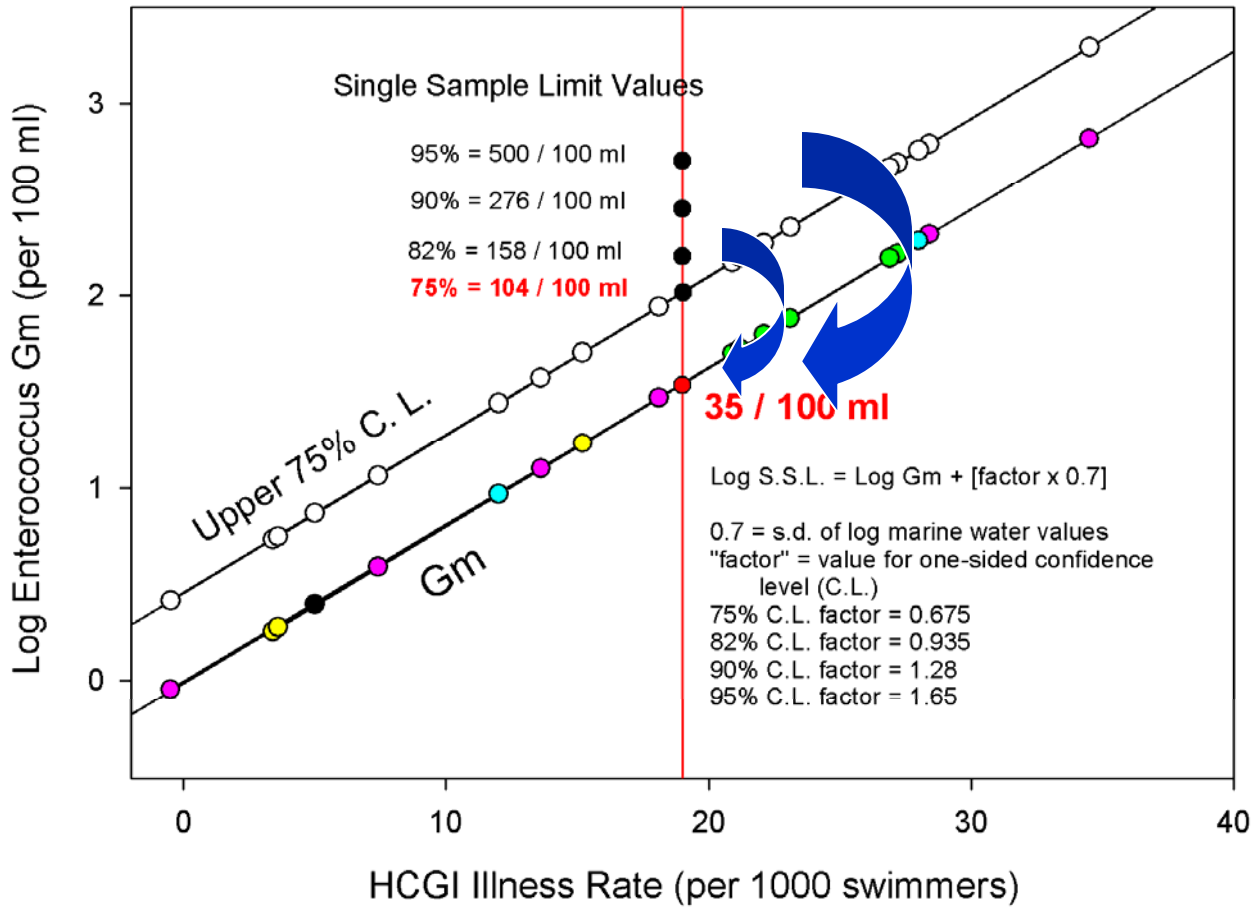
EPA 1986 Criteria: Marine Waters (con't)

- Enterococci best correlation with HCGI illness (NY: r = 0.96).
  - NY: Total coliforms (r=0.65); Fecal coliforms (r=0.51); E. coli (r=0.56).
  - Based on clustered data.
- Fecal coliform Gm of 200 = Enterococcus Gm of 35 in these studies.
- Enterococci: 35 / 100 ml = 19 illnesses/1000 bathers (~ 1 per every 50 swimmers).
- Thus, the 1986 "acceptable risk level" was based on the historically accepted risk, but it is still arbitrary insofar as the historical risk was itself arbitrary."

Enterococcus Density vs. Highly Credible Gastrointestinal (HCGI) Illness Rate



EPA 1986 Marine Water Criteria: Enterococcus Geometric Mean (Gm) and Single Sample Limit (S.S.L.) Values for a Highly Credible Gastrointestinal (HCGI) Illness Rate of 19 per 1000 Swimmers



Upper C.L.s are arbitrarily derived percentage values.

TABLE 3 - COLIFORM VARIATIONS OVER 12-HOUR PERIODS

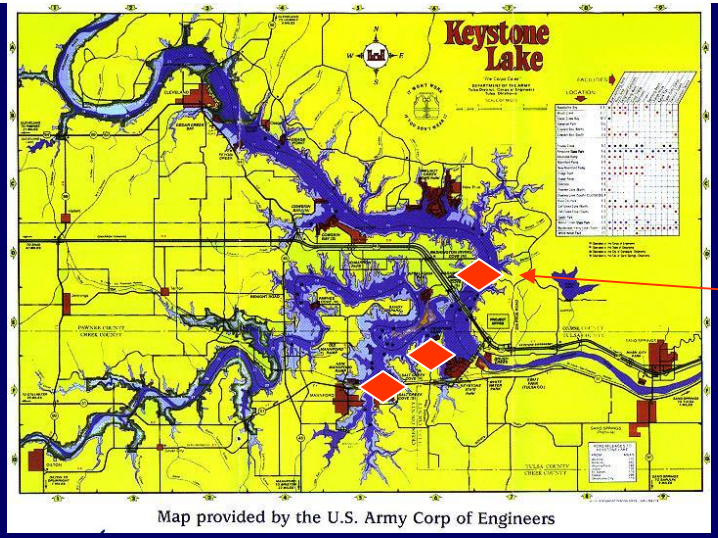
Date	Sample Point	Coliform MPN per 100 ml						
		9 AM	11 AM	1 PM	3 PM	5 PM	7 PM	9 PM
		North Beach						
7/13	9	930	1,500	2,400	2,400	4,600	11,000	1,500
	83	930	2,400	930	910	2,400	230	930
7/18	9	230	4,300	4,300	750	2,400	930	930
	83	230	2,400	230	91	93	460	460

Distance between sample points - 2,000 feet

EPA 1986 Criteria: Fresh Waters

1986: Halley's comet returns

- 2 Valid Indicators:
  - Enterococcus Gm of 33/100 ml (s.s.limit 61-151/100ml) OR
  - E. coli Gm of 126/100 ml (s.s. limit of 235-576/100 ml).
- Based on epi. studies from 1978-1982
  - Keystone Reservoir near Tulsa, OK (3 beaches; 79, 80).
  - Lake Erie at Erie, PA (2 beaches; 79, 80, 82).
- Freshwater S.S.L. numbers based on s.d. log data = 0.4.
  - Note: s.d. log value for NJ lakes, based on 8-yr FC data = **0.7** not 0.4. Rivers too.



Keystone Lake, OK:

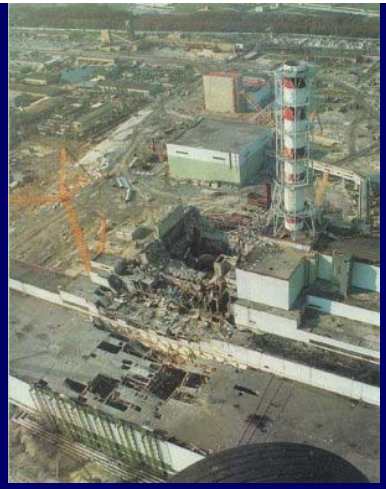
Arkansas & Cimarron Rivers dammed; 26,000 acre lake; 15 miles west of Tulsa, OK; 3 beaches

Salt Creek (N), Keystone Ramp, Wash. Irving Cove (S)

Presque Isle & Erie, PA

Presque Isle State Park, Erie, PA; 2 beaches

EPA 1986 Criteria: Fresh Waters



1986: Chernobyl blew up

- Enterococci (r=0.74) and E. coli (r=0.80) equally good predictors of GI illness.
- Fecal coliforms: no correlation to GI illness (r= -0.08).
  - BUT: Lake Erie Gms: FC = 37, 104, 60; E. coli = 137, 236 & 146. Data suspect at Lake Erie.
- Slope based on 9 data points.
- Fecal coliform Gm of 200 (per 100 ml) = Enterococcus Gm of 33 and E. coli Gm of 126 in these studies.
- Equal to illness rate of 8 per 1000 bathers (1 per 125).

NJ Selected E. coli instead of Enterococcus for future freshwater monitoring. Why?

1. E. coli comprise the majority of fecal coliform bacteria – can better estimate long-term WQ trends than if Enterococcus were monitored. (Also, can better extrapolate FC standard deviation values to E. coli s.d. values).
2. Based on NJ monitoring data, there will be a smaller increase in SWQ exceedences using E. coli than using Enterococcus (~11% vs. ~25%).
3. Analysis of non-US studies revealed that, for fresh waters, E. coli was a more consistent predictor of GI illness than enterococci and other indicators (T.J. Wade et al. 2003. Env. Health Persp. 111: 1102-09).

NJ Uses of the 1986 Criteria

- Surface Water Quality
  - Ambient monitoring (305b/303d report/list)
  - Future Pathogen TMDLs ?
    - “CA regional water authority will fine cities surrounding Santa Monica Bay up to $10,000 a day if the beach water does not meet the standards.” Yikes – stop the rain, wind and birds!!
  - Wastewater discharge permit limits (NPDES).
    - Molecular assays measure DNA from live AND dead or non-infectious cells.
- Bathing Beach standards (DHSS regs.).

Current Indicator Test Delay Problem: 2006 Example

- Beachwood Beach, Beachwood, Ocean Co.
  - Monday 8/14: sampled; beach open.
  - Tuesday 8/15: Mon count > 104; beach open; resampled.
  - Wed 8/16: Tues count > 104; beach closed, resampled.
  - Thurs 8/17, Wed count < 104, beach opened.
- We can only tell what the water was like yesterday.

So What's Next?

- CWA Section 304(a)(9): EPA supposed to formulate new or revised water quality criteria for pathogens by 2005, based on research required under Section 104(v).
- They didn't make it.
- NRDC has threatened a lawsuit, so EPA looking for "a better mousetrap" within a 3-4 year timeframe.

CWA Section 104(v)-specified Research

Cepheid Smart Cycler

- 2003, 2004 EPA Freshwater Epidemiological study ("NEEAR")(*)
  - 3 beaches on Lake Michigan and 1 on Lake Erie.
    - West Beach, Indiana Dunes, IN [03], Huntingdon Beach, Bay Village, OH [03], Wash. Park, Michigan City, IN [04], and Silver Beach, St. Joseph, MI [04].
    - Point source (POTW) impact.
  - Enterococcus (culture Method 1600) and qPCR Entero. and qPCR Bacteroides ("real-time" molecular assays). E. coli not monitored!
    - qPCR assays are 2-hour assays (vs. 24 h for culture assays). Ent. rRNA probe.
  - Positive correlation between qPCR Entero. levels and bather HCGI illness. 1 log increase in qPCR Entero-CE (**) associated with 1.37 [1.1-1.7] increase in the odds of contracting GI illness.
    - Trends not observed for non-GI illness endpoints.
  - Inter-sample precision was poor for Entero. qPCR at ambient densities.

- Marine water study (2005) halted due to Hurricane Katrina.
  - Biloxi, Mississippi bathing beach no longer exists. Study now slated for 2007.

* T.J. Wade, et al. 2006. Env. Health Persp. 114: 24-28 and R.A. Haugland et al. Wat. Res. 39: 559-68.

** CE = Cell equivalents.

Avoiding Time-Delay Problem
Approach 1: "real-time" PCR

Applied Biosystems 7900HT

- Rapid molecular assays (2 hr & shorter assays).
- Can use on any organism or group of organisms (if probes available).
- Enterococcus qPCR correlated with bather illness, using Ent rRNA target probe.
- But potential implementation issues:
  - PCR enzyme inhibition, DNA cross-contamination, how much QC, lab capability and capacity, cost?
  - With respect to real-world monitoring protocols, how much time is saved ?

Avoiding Time-Delay Problem: Approach 2. "Annapolis Protocol"(*)

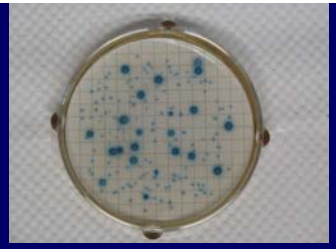
Pres. Jimmy Carter

- Beach classification scheme
- Name derived from location of 1998 EPA-sponsored WHO-organized meeting of experts.
- No ongoing monitoring/open-or-close decisions required.
- Beach ranked by several criteria:
  - Microbial quality rank, based on indicator 95th percentile concentration (not Gm). May include non-microbial or additional indicators as well.
  - Sanitary survey analysis rank (potential exposure to sewage impact, river impact, & bather density).
  - Overall matrix rank (poor, fair, good, excellent).
  - Includes management & monitoring options based on ranking.
- Many elements require substantial sampling and/or testing to verify.
- NJDEP (Dave Rosenblatt) was a participant.

* Guidelines for safe recreational water environments. WHO, Geneva, 2003; WHO/SDE/WSH/99.1, Geneva, 1999.

Avoiding Time-Delay Problem
Approach 3: Predictive Modeling

- Predicting site-specific indicator concentration (or exceedence) in advance, based on factors shown to influence such levels - rainfall, turbidity, wind, wave-height, tide, sunlight, etc.
- Least squares regression models, artificial neural networks, other?
- Predictive models yield more "correct responses" (better predict exceedences) than current methods.
- Useful 7 days/week (not just sampling day).
- Labor/time/cost-intensive but can focus on "problem beaches" only.
- Can help track microbial contaminant sources.



Enterococcus

Method 1600

Possible Future Directions

(topics for March, 2007 EPA workshop)

Colilert (R)
Idexx, Inc.

- Measure pathogens directly?
  - Pathogen DNA-on-a-chip, microfluidic systems, immunoassays, qPCR, other?
- Use other or multiple indicators?
  - *C. perfringens*? (Hawaii finds useful) Coliphages? Bacteroides phages? Adenovirus and/or polyomavirus?
- Different indicators for different purposes?
  - Bathing beaches vs. SWQ monitoring.
  - Tropical vs. temperate climates.
  - Primary contact vs. shellfish harvest.
- Employ non-biological indicators?
  - Caffeine, sterols, detergent components, pharms & PCPs, etc.

Related Issues


Lake Hopatcong

- Risk of animal vs. human pollution.
  - Epi studies were done in places with mixed pollution sources.
- Publicly acceptable risk level?
  - Is there such a thing?
  - Are current levels OK?
    - e.g., Delaware and Maine use risk levels lower than 19/1000.
- Risk to sensitive sub-populations?
- Risk in flowing rivers vs. lakes?
- Statistical approach for epi studies: linear or logistic regression or other? Data handling: parametric or non-parametric methods?
  - Amount of illness vs. probability of illness.
  - Detectable risk or acceptable risk?
    - No observed adverse effect level (NOAEL).
    - Recent German freshwater epi. study: NOAEL when E. coli < 100 (per 100 ml) or Enterococcus < 25.
- Are secondary contact criteria necessary?
- Can recreational criteria protect drinking water sources and shellfish harvest waters?