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Waterborne Pathogens and their Indicators

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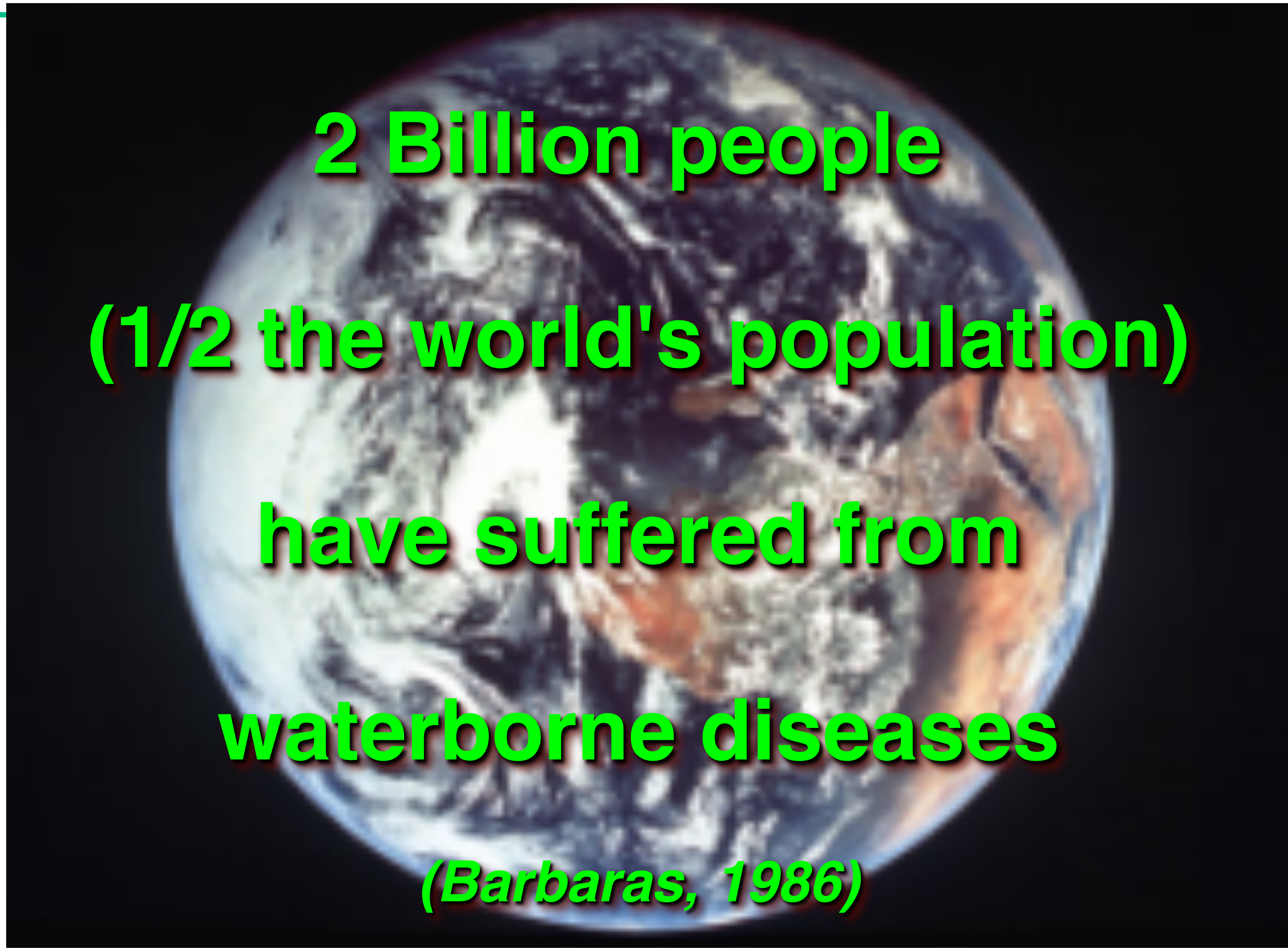
2 Billion people
(1/2 the world's population)
have suffered from
waterborne diseases
(Barbaras, 1986)

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Incidence of Waterborne Disease

Worldwide: 250 million new cases each year

50,000 per day

20 million deaths

USA: 30 outbreaks of 100,000 cases per year

One in Four Hospital Beds Worldwide is occupied by someone who drank unsafe water.

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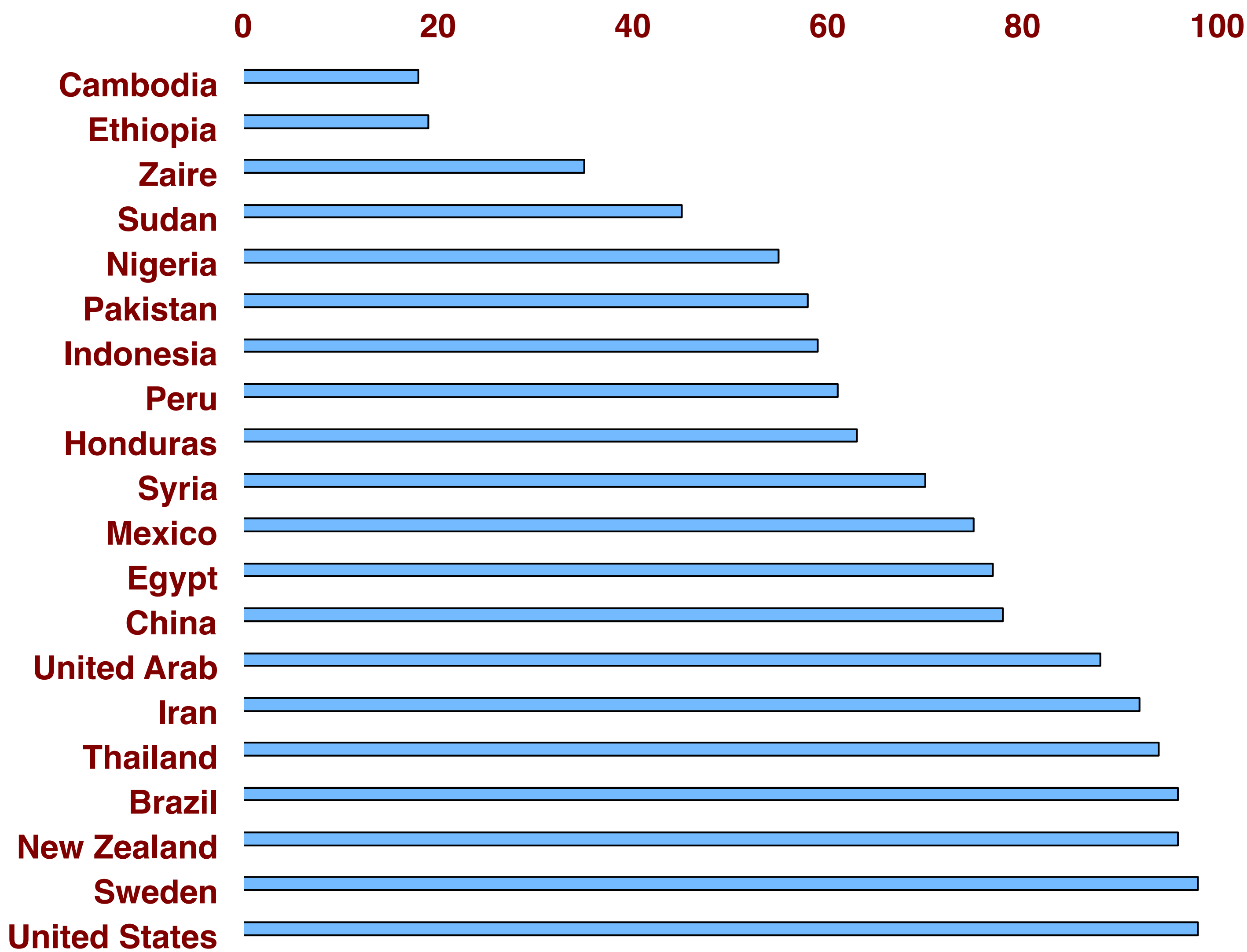
POPULATIONS WITH ACCESS TO SAFE DRINKING WATER (PERCENT OF POPULATION)



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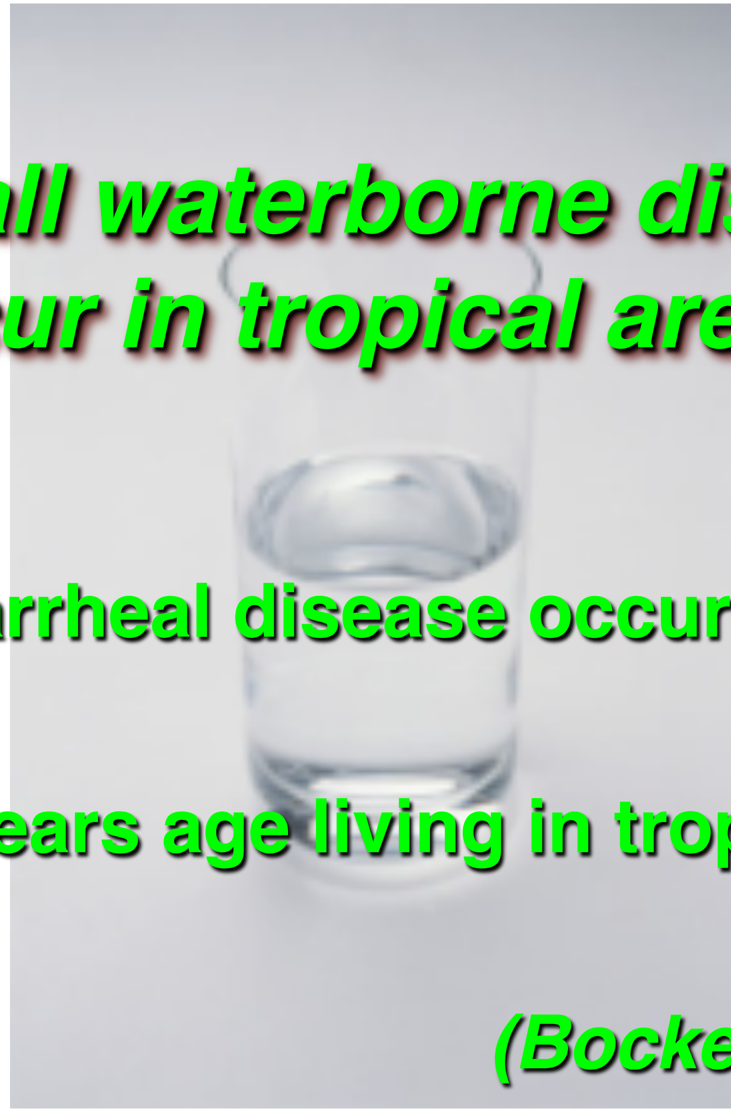


75% of all waterborne diseases occur in tropical areas

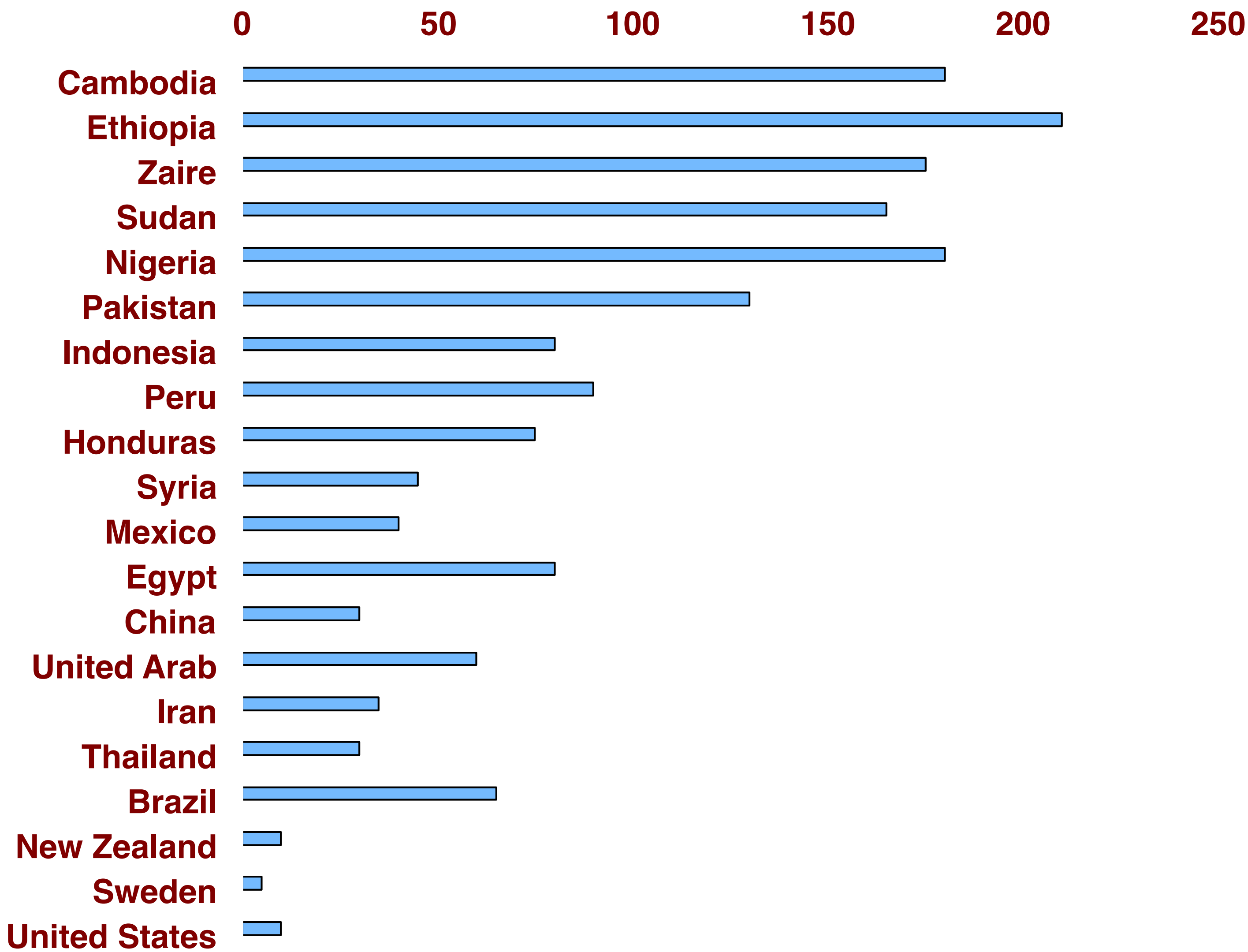
50% of all diarrheal disease occur in children

< 5 years age living in tropics

(Bockemühol, 1985)

WATER QUALITY AND CHILD SURVIVAL

Deaths of Children Under 5 Years of Age (deaths per thousand)



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ECONOMIC IMPORTANCE OF WATERBORNE DISEASE

- Last 10 years > $600 billion worldwide
- 1 outbreak in the USA > $340,000
- $1.1 billion in USA during the next 10 years
- $400 million during the next year

WHO (1985)

Major Differences between Tropical and Temperate Areas

1. Physical and Chemical
2. Biological
3. Social and Economic

Physical and Chemical

1. **Temperature**
   A. Higher (usually not below 18°C)
   B. More Constant (20-30°C range)
   C. Causes permanent lentic stratification (oligomictic)
2. **Dissolved Oxygen**
   A. Higher temperatures lowers solubility
   B. Greater fluxes
3. **Light**
   A. Higher intensity
   B. Less change in length of day during year
4. **Water**
   A. Usually higher annual rain fall
   B. Catastrophic rain fall (eg. 61 cm in 24 h)
   C. "Seasons" usually delineated by rain fall, more variable in length and less predictable

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Biological

1. Higher light intensity and temperature cause higher productivity
2. Lack of seasons allows reproduction, senescence and input of degradative substances to occur year round
3. Higher faunal and floral diversity
4. Different flora and fauna
5. Higher microbial diversity (Hill and Rai, 1982)
6. Domination of mesophiles and thermotolerant microbes
    - A. Tropics: highest plate count at 30°C for 72 h (Jen & Bell, 1982)
    - B. Temperate: highest plate count at 22°C for 72 h (Holden, 1970)
7. More waterborne diseases

Social and Economic

1. 65% of world lives in tropics but they have only 10% of wealth
2. Poor
   A. Lack of public education
   B. No research and development
   C. More preventable diseases, fewer hospitals and trained personnel
3. Greater reliance on unprotected, untreated traditional water sources in part due to cultural norms and taboos
4. Unrealistic standards developed by temperate nations
   A. Damages national pride
   B. Damages world opinion
   C. Puts unnecessary demands on very limited and needed resources

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If cycles are not broken these under developed nations become "never-to-be-developed" nations

DENSITY OF INDICATORS AND PATHOGENS IN NATURAL TROPICAL WATERS

Site	TC	FC	EC	FS	Pathogens	Reference
Ceylon	$>10^3$		$>10^3$	0		Evison & James (1973)
India	$>10^3$		$>10^3$	0		Evison & James (1973)
Singapore		$>10^3$	$>10^3$	0		Evison & James (1973)
New Guinea	$0\text{-}10^4$			$0\text{-}10^4$		Feachem (1974)
Sierra Leone	$40\text{-}10^5$	$30\text{-}10^5$	$7\text{-}10^4$	0		Wright (1982)
Hawaii		$10^2\text{-}10^4$	$10^2\text{-}10^4$			Fujioka & Shizumura (1985)
Nigeria	$8\text{-}10^5$					Oluwande et al. (1983)
Botswana	0	0	0	10^3		Thomson (1981)
Ivory Coast		10^4				Lavoie & Viens (1983)
Puerto Rico	10^6	10^6	10^5	10^4	0	Hazen et al. (various)

TC = total coliforms, FC = fecal coliforms, EC = E. coli, FS = fecal streptococci.

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OTHER INDICATORS

- **Fecal Coliforms - more specific than total coliforms, since they rely on the thermal tolerant nature of E. coli; however, the target, E. coli, is not appropriate.**
- **E. coli - more specific than fecal coliforms but subject to the same limitations as total coliforms and fecal coliforms, since the target is E. coli.**
- **Coliphages - more specific than E. coli and avoid the problems associated with media growth of E. coli; however, since they are based on a host that may be a poor target they also would be a poor target. Male specific phages or those that could be shown only to multiply in E. coli while it is in the GI tract could be appropriate.**
- **Fecal Streptococci - appear to be as specific as E. coli; however, they also appear to survive in a manner similar to E. coli. Specific strains may be used to indicate animal vs. human contamination.**
- **Candida albicans - Poor prospects. Survives indefinitely in both marine and freshwater environments. Media does not readily differentiate species.**
- **Obligate Anaerobes - Good prospects. Inability to survive in natural waters makes these good candidates, Clostridium perfringens has promise; however, it may be found in soil, Bifidobacteria also show promise, but media needs much work, Bacteriodes fragilis phages show great promise, but more testing is needed.**
- **Chemicals: 5-ß-coprostanol, Caffiene**
- **Microarrays for pathogens**

Climate Change

- Water scarcity compromise hygiene as dwindling resources become increasingly polluted. In addition, reduced water pressure in distribution systems increases the risk of back siphoning of contaminated water.
- Floods can lead to breaching of barriers between sewage and water systems.
- Warming (or cooling) trends result in changes in the distribution of pathogens and disease vectors, e.g., the coastal algal blooms that precede cholera epidemics in Bangladesh (McMichael 1993; Garrett 1994; Huq, West, Small, et al., 1984).
- Increased exposure to ultraviolet rays (UV) may result in increased susceptibility to disease (Chapman, Cooper, Defabo, et al. 1995), and in increased mutation rates, with unpredictable effects on the ecosystem as a whole and on pathogen development specifically.

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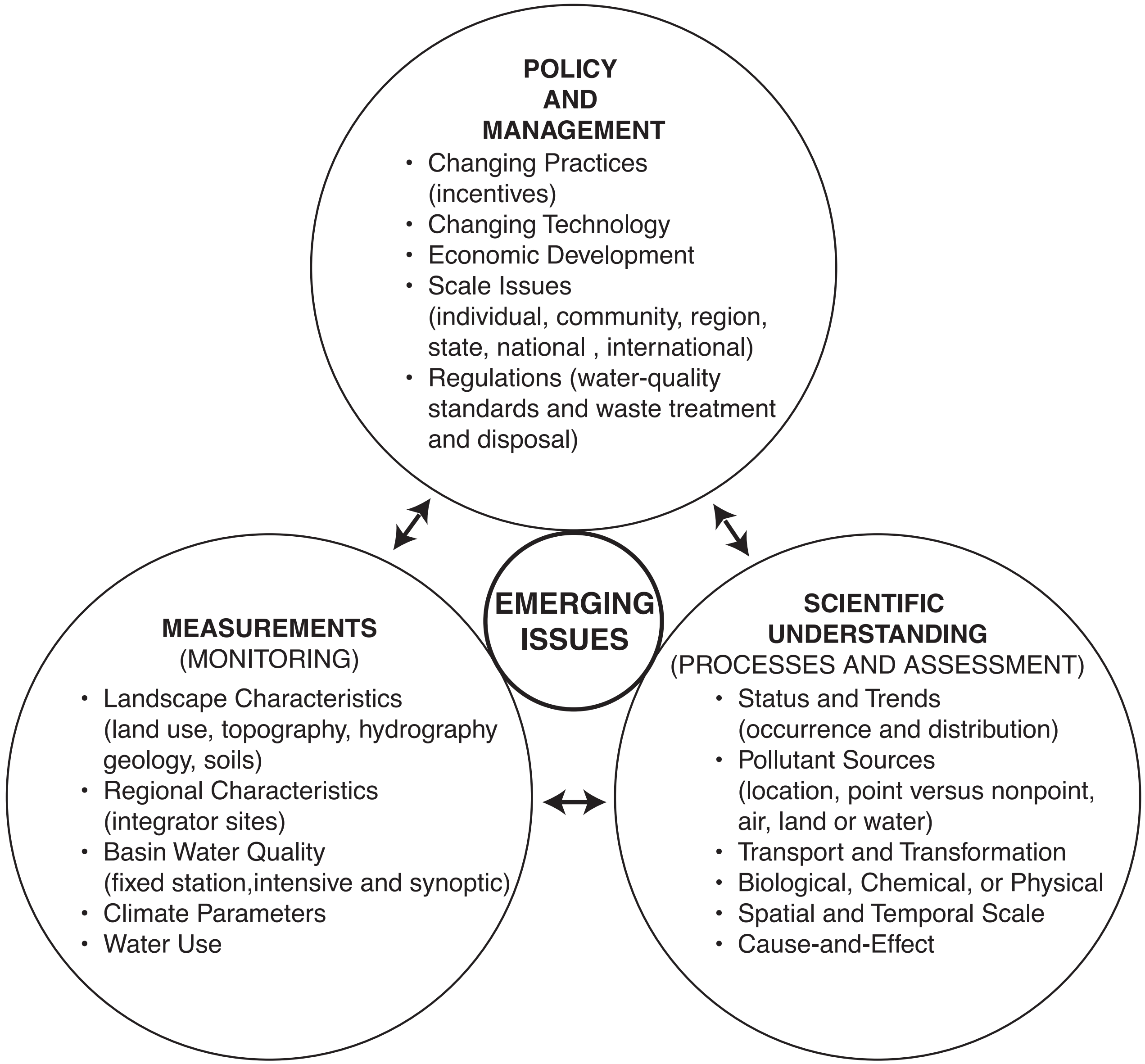
Pathogens

- Conventional water treatment processes may not reliably prevent diseases caused by newly recognized agents, e.g. *Cryptosporidium*.
- Microorganisms with a higher level of resistance to chemicals used in water treatment and disinfection than expected have been recently recognized, including *Cryptosporidium, Giardia, Cyclospora*, and the mycobacteria (Ford 1993; Soave and Johnson 1995).
- Pathogens possess survival mechanisms that protect them from disinfections, such as the rugose form of *Vibrio cholerae* (Morris, Johnson, Rice, et al. 1993) (wrinkled, dwarf forms on culture plates that retain full virulence or *Legionella pneumophila* that multiplies in amoebae where it lies protected from disinfection (Kilvingto and Price 1990).
- As further research is done on transmission routes of infectious diseases, particularly for an increasingly large immunologically compromised population, many other infectious agents will be discovered to be transmissible by water.
- Declining immunities to many pathogens, mainly due to better sanitary conditions, increases the susceptibility and risk of disease during system failures.
- Development of antibiotic resistant strains of waterborne pathogens.

Global Water Quality Issues



Recreational Water MCL

Current MCL: 1000 Coliforms

200 Fecal Coliforms

Recommended: Marine - 35 Enterococci

Fresh - 126 *E. coli*

33 Enterococci

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Drinking Water Treatment Problems

1. Increased resistance of bacteria and viruses attached to activated carbon finings
2. Increased resistance of certain pathogens eg. Giardia lamblia, Coxsackie virus
3. Increased resistance of bacteria and viruses attached to clay particles ie. turbidity
4. Decreased disinfection when organic and nitrogen levels in the water are high eg. 40 times more chlorine may be required
5. Injury of bacteria which only effects recovery

Direct Detection of Pathogens

1. Fluorescent Antibodies (DFA, IFA)
2. ELISA
3. RIA
4. Probes (DNA, RNA)

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Problems assessing microbiological water quality in the Tropics

1. Regrowth of indicators in freshwater.
2. Thermotolerant and mesophilic background flora over grow assays which rely upon the thermotolerant nature of indicators and a psychrophilic background flora.
3. High false-positive natural flora (unknown species) using media techniques.
4. Pathogens found in the absence of indicators, eg. Salmonella spp.
5. Higher natural productivity of environments, allows greater survival and regrowth of indicators and pathogens.
6. Different flora and fauna of tropics, creates different interactions and environments for indicators and pathogens.
7. Torrential rainfall, in some areas causes extreme changes in allochthonous input of nutrients and microbes from surrounding environments.
8. Greater number of waterborne diseases.
9. Greater reliance on local surface waters as traditional drinking water sources.
10. Few sewage treatment and water treatment facilities and inadequate monitoring and enforcement.
11. Possible autochthonous origin of indicators.
12. Because of higher annual temperatures people spend more time in direct contact with water.

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Bifidobacteria

1. Found in all humans from birth.
2. Not common in other animals.
3. Obligate anaerobes, ie. do not survive extraenterally.
4. Easy to enumerate on YN-6 media.
5. Not known to exist in any natural environment.

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Fecal Coliform Positive Bacteria

Escherichia coli

Enterobacter spp.

Klebsiella pneumoniae

Citrobacter spp.

rare: Vibrio spp., Aeromonas hydrophila

Commonwealth of Puerto Rico

Regulation of the Secretary of Health Number 50, Approved 21 June 1983

Provisions of Law 5, 21 July 1977

Repealing Rsh Number 44, 29 November 1979

Article VI. Primary Drinking Water Regulations

6. MCL for Microbes

EPA Proposed Regulations

EXISTING

1 COLIFORM COLONY/100 ML MONTHLY AVERAGE

4 COLIFORM COLONIES/100 ML FOR A SINGLE SAMPLE

PROPOSED

PRESENCE OF ANY COLIFORMS

REPORT ALL FOLLOW UP SURVEYS IF COLIFORMS PRESENT

* FEDERAL REGISTER 10/5/83

Federal Law

SAFE DRINKING WATER ACT (PUBLIC LAW 93-523)

APPROVED 24 JUNE 1977 EPA

U. S. PUBLIC HEALTH SERVICE 1962,1915

U. S. STANDARDS ESTABLISHED IN 1914

Total Coliform Viable Counts

	Elevation		
Above Ground (m)	250	350	700
0-2	13,400	23,800	46,500
2-4	11,100	2,890	5,670
4-6	22,800	5,330	21,200

* CFU mL^{-1}

Indicator and Pathogen Survival in situ

		Density	Survival (h)*	Reference
E. coli	Temp	10^9	50	Gordon and Fliermans (1978)
		10^5	30.6	McFeters et al. (1974)
	Trop	10^7	∞	Carrillo et al. (1985)
		10^7	294	López et al. (1987)
		10^6	206	Valdes-Collazo et al. (1987)
S. faecalis	Trop	10^7	226	Muñiz et al. (1988)
B. adolescentis	Trop	10^7	92.6	Carrillo et al. (1985)
V. cholerae	Temp	10^5	13	McFeters et al. (1974)
	Trop	10^7	198	Pérez-Rosas (1983)
Y. enterocolitica	Trop	10^8	124	Elías et al. (1988)
C. albicans	Trop	10^6	∞	Valdés-Collazo et al. (1987)
A. hydrophila	Temp	10^5	∞	McFeters et al. (1974)
		10^7	75	Hazen and Esch (1983)
		10^8	100	Fliermans et al. (1977)
	Trop	10^7	∞	Hazen et al. (1982)
S. typhimurium	Temp	10^5	28.8	McFeters et al. (1974)
	Trop	10^8	131	Jiménez et al. (1987)
K. pneumoniae	Trop	10^6	125	López et al. (1987)
P. aeruginosa	Trop	10^5	∞	Cruz (1987)

*survival time (T90)= time to reach 90% reduction of initial cell density

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Fecal Coliform Assay Methods

Parameter	MMB Temp	MMB Trop	m-FC1 Temp	m-FC1 Trop	mFC2 Temp	mFC2 Trop	mTEC Temp	mTEC Trop
Accuracy[a]	59	67	89	84	100	105	94	93
Specificity[b]								
False positive	11	39	16	30	18	19	13	36
Undetected	4	25	1	20	2	11	2	21
Selectivity[c]	88	66	85	72	90	82	86	66
Comparability[d]								
FC recovery	26	41	41	75	48	94	45	73
Non-FC recovery	4	30	11	29	15	19	7	18
Overall rank[e]	2	4	3	2	1.5	1	1.5	3

* All data labelled temp is from Pagel et al. (1981), all data labelled trop is from Santiago-Mercado and Hazen (1987)

[a] Mean percentage number of colonies on test medium/[mean number of colonies on a non-selective medium] X 100, using E. coli (ATCC 10798 and ATCC 23848). b Percentage false positive error = false positive error + number of presumptive target colonies - number of verified target colonies/[total presumptive target colonies] X 100. Percentage false negative error = False negative counts/[verified + undetected target counts] X 100. c Selectivity Index = Presumptive typical colonies/[presumptive typical or target colonies + presumptive non-typical or non-target Colonies] X 100. d Percentage fecal coliform (FC) recovery and percentage non-fecal coliform (NFC) recovery. e Best overall efficiency of the method is given by the lowest overall rank.

Fecal Streptococci

1. Gram Positive
2. Coccus Shaped
3. Resistant to Sodium Azide
4. Ratios of FS/FC > 4 Indicate Human Contamination

Media: M-Enterococcus Agar with TTC 35°C for 48 H

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Quantitation of Bacteria

1. **Direct Count**
2. **Viable Count**
3. **Activity**
   - A. Respiration
   - B. protein synthesis
   - C. nucleic acid synthesis
   - D. uptake of substrates
   - E. adenylate concentration
4. **Biomass**
5. **Pathogenicity?**

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Measuring Environmental Activity

1. respiration (INT reduction)
2. protein synthesis (AODC, radiolabel)
3. nucleic acid synthesis (Thymidine uptake, MA)
4. uptake of carbon sources (radiolabeled glutamate)
5. adenylate concentration (luciferin-luciferase)
6. Pathogenicity and/or toxicity (LD50, cytoxicity)

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Good Indicator Assumptions

1. it must be present whenever the pathogens concerned are present.
2. it must be present only when the presence of pathogenic organisms is an imminent danger.
3. it must occur in much greater numbers than the pathogens
4. it must be more resistant to desinfectants and to the aqueous environment than the pathogens.
5. it must grow readily on relatively simple media.
6. it must yield characteristics and simple reactions enabling, as far as possible, an unambiguous identification of the group or species.
7. it should preferably be randomly distributed in the sample to be tested, or it should be possible to obtain a uniform distribution by simple homogenization procedures.
8. its growth in artificial media must be largely independent of any organism present, i. e. the growth of indicator bacteria should not be seriously inhibited by the presence of other species.

(Bonde, 1978)

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Waterborne Viral Diseases

Organism	Disease	Infectious Dose
ADENOVIRUS	enteritis, pharyngitis	1 PFU
CALICIVIRUS	enteritis	1 PFU
NORWALK VIRUS	enteritis	1 PFU
CORONAVIRUS	enteritis	1 PFU
COXSACKIEVIRUS A	meningitis	1 PFU
COXSACKIEVIRUS B	meningitis	1 PFU
ECHO VIRUS	enteritis, meningitis	1 PFU
*HEPATITIS A VIRUS	hepatitis	1 PFU
*POLIOVIRUS	poliomyelitis	1 PFU
REOVIRUS	enteritis	1 PFU
*ROTAVIRUS	enteritis	1 PFU
ASTROVIRUS	enteritis	1 PFU

**Found in Puerto Rico*

Waterborne Helminthe Diseases

Organism	Disease	Infectious Dose
***Schistosoma mansoni**	schistosomiasis	1 cercariae
S. haemotobium	schistosomiasis	1 cercariae
S. japonicum	schistosomiasis	1 cercariae
S. intercalatum	schistosomiasis	1 cercariae
S. mekongi	schistosomiasis	1 cercariae
**Fasciola hepatica*	fascioliasis	1 metacercariae
Paragonimus westermani	paragonimiasis	1 metacercariae
Clonorchis sinensis	chinese liver fluke	1 metacercariae
Diphyllobothrium latum	pernicious anaemia	1 pleurocercoid

**Found in Puerto Rico*

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Other Waterborne Diseases

Organism	Disease	Infectious Dose
Cyanobacteria		
Anabaena spp.	neuromuscular poison	?
Aphanizomenon spp.	neuromuscular poison	?
Microcystis spp.	neuromuscular poison	?
Fungi		
**Candida spp.*	Candidiasis	?
Rhinosporidium seeberi	Rhinosporidiosis	?
Protozoa		
Cryptosporidia	cryptosporidiosis	?
**Giardia lamblia*	giardiasis	1 cyst
**Entameba histolytica*	dysentery	1 cyst
**Naegleria fowleri*	meningoencephalitis	?
Acanthamoeba spp.	meningoencephalitis	?

**Found in Puerto Rico*

Waterborne Bacterial Diseases

Organism	Disease	Infectious Dose
Acinetobacter calcoaceticus	Noscomial	NF
**Aeromonas hydrophila*	Enteritis, Wounds	?
Aeromonas sobria	Enteritis, Wounds	?
Aeromonas caviae	Enteritis, Wounds	?
Chromobacterium violaceum	Enteritis	?
**Citrobacter spp.*	Nosocomial	?
**Enterobacter spp.*	Nosocomial	?
**Escherichia coli serotypes*	Enteritis	>10^6 CFU
Flavobacterium meningosepticum	Nosocomial, Meningitis	?
Francisella tularensis	Tularemia	10 CFU
Fusobacterium necrophorum	Liver abscess	10^6 CFU
**Klebsiella pneumoniae*	Nosocomial, Pneumonia	?
Leptospira icterohaemorrhagia	Leptospirosis	?
**Legionella pneumophila*	Pontiac Fever, Legionellosis	>10 CFU
**Morganella morganii*	Urethritis, Nosocomial	?

**Found in Puerto Rico*

Waterborne Bacterial Diseases

Organism	Disease	Infectious Dose
*Mycobacterium tuberculosis	Tuberculosis	?
Mycobacterium marinum	Swimming pool granuloma	?
Plesiomonas shigelloides	Enteritis	?
Pseudomonas pseudomallei	Meliodosis	?
*Salmonella enteritidis	Enteritis	>10^6 CFU
*Salmonella montevideo B	Salmonellosis	?
Salmonella paratyphi A & B	Paratyphoid Fever	?
*Salmonella typhi	Typhoid Fever	10^5 CFU
*Salmonella typhimurium	Salmonellosis	>10^5 CFU
*Serratia marcescens	Nosocomial	?
*Staphylococcus aureus	Abscesses, Food poisoning	NF
Vibrio alginolytcus	Wounds	?
*Vibrio cholerae	Cholera dysentery	10^3 CFU
*Vibrio parahaemolyticus	Enteritis	10^5 CFU
*Vibrio vulnificus	Wounds	?
*Yersinia enterocolitica	Enteritis	?

*Found in Puerto Rico