



AMERICAN WATER

Development of a *Legionella* Risk Management Strategy

Zia Bukhari, Ph.D.

WaterReuse-12-05 Project Team

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Microbes

Taylor *et al.* (2001) identified 1415 species, representing 636 genera for their public health significance

Category	Total genera screened	PubMed*	Biosis	Web of Science	Total of PubMed, Biosis, and Web of Science
Bacteria	140	Coliform ^a (18) <i>Escherichia</i> (10) <i>Clostridium</i> (2) <i>Enterococcus</i> (2) <i>Legionella</i> (2) <i>Photobacterium</i> (2) <i>Aeromonas</i> (1) <i>Alcaligenes</i> (1) <i>Bacteroides</i> (1) <i>Citrobacter</i> (1) <i>Helicobacter</i> (1) <i>Mycobacterium</i> (1) <i>Salmonella</i> (1) <i>Staphylococcus</i> (1) <i>Vibrio</i> (1) <i>Yersinia</i> (1)	Coliform ^a (18) <i>Escherichia</i> (11) <i>Salmonella</i> (6) <i>Clostridium</i> (5) <i>Legionella</i> (4) <i>Enterococcus</i> (3) <i>Shigella</i> (3) <i>Bacillus</i> (2) <i>Mycobacterium</i> (2) <i>Pseudomonas</i> (2) <i>Streptococcus</i> (2) <i>Aeromonas</i> (1) <i>Bacteroides</i> (1) <i>Campylobacter</i> (1) <i>Citrobacter</i> (1) <i>Helicobacter</i> (1) <i>Photobacterium</i> (1) <i>Staphylococcus</i> (1) <i>Yersinia</i> (1)	<i>Escherichia</i> (21) Coliform ^a (15) <i>Clostridium</i> (6) <i>Legionella</i> (4) <i>Bacteroides</i> (3) <i>Salmonella</i> (3) <i>Campylobacter</i> (1) <i>Citrobacter</i> (1) <i>Helicobacter</i> (1) <i>Mycobacterium</i> (1) <i>Photobacterium</i> (1) <i>Vibrio</i> (1)	Coliform ^a (51) <i>Escherichia</i> (42) <i>Clostridium</i> (13) <i>Salmonella</i> (10) <i>Legionella</i> (10) <i>Enterococcus</i> (5) <i>Bacteroides</i> (5) <i>Mycobacterium</i> (4) <i>Citrobacter</i> (3) <i>Helicobacter</i> (3) <i>Photobacterium</i> (3) <i>Shigella</i> (3) <i>Aeromonas</i> (2) <i>Bacillus</i> (2) <i>Campylobacter</i> (2) <i>Pseudomonas</i> (2) <i>Staphylococcus</i> (2) <i>Streptococcus</i> (2) <i>Vibrio</i> (2) <i>Yersinia</i> (2) <i>Alcaligenes</i> (1)
Fungi	130	None reported	<i>Aspergillus</i> (1) <i>Cunninghamella</i> (1) <i>Prototheca</i> (1)	None reported	<i>Aspergillus</i> (1) <i>Cunninghamella</i> (1) <i>Prototheca</i> (1)
Helminthes	136	None reported	<i>Trichuris</i> (1)	<i>Trichuris</i> (1)	<i>Trichuris</i> (2)
Protozoa	25	<i>Cryptosporidium</i> (13) <i>Giardia</i> (9)	<i>Cryptosporidium</i> (31) <i>Giardia</i> (21)	<i>Cryptosporidium</i> (27) <i>Giardia</i> (20)	<i>Cryptosporidium</i> (71) <i>Giardia</i> (50)
Viruses (and prions)	205	Enterovirus/Poliovirus (14) Hepatitis A (1) Noroviruses (1)	Enterovirus/Poliovirus (8) Hepatitis A (2) Rotavirus (2)	Rotavirus (3) Enterovirus/Poliovirus (1) Hepatitis A (1)	Enterovirus/Poliovirus (23) Rotavirus (5) Hepatitis A (4) Noroviruses (1)

Microbes of interest

- Exhaustive literature review
 - >1400 microbes (representing >600 genera)
- Different databases (Biosis, PubMed & Web of Science)
- Selected 20 based on:
 - Citations,
 - Difficulty of treatment,
 - Public health significance,
 - USEPA CCL3 list and
 - Project Advisory Committee input

Bacteria

- Coliforms
- *Clostridium*
- Enterococci
- *E. coli* (Non-pathogenic)
- *E. coli* (pathogenic)
- *Helicobacter*
- *Legionella*
- *Mycobacterium*
- *Salmonella*
- *Shigella*

Protozoa & viruses

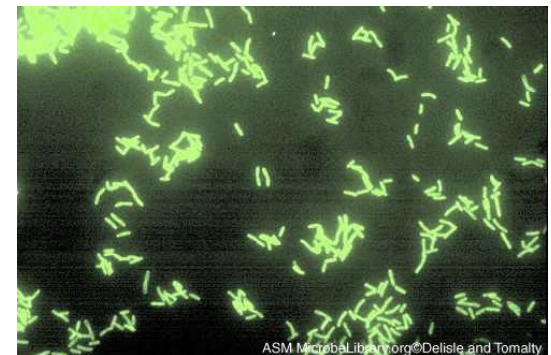
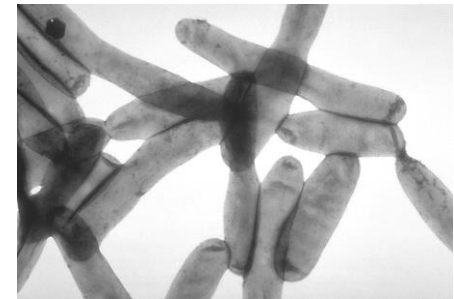
- *Acanthamoeba*
- *Cryptosporidium*
- *Giardia*
- *Naegleria*
- Enterovirus
- Hepatitis A
- Noroviruses
- Rotavirus
- Bacteriodes phage
- Coliphage

Significance of Microbes

Category	Organism (group)	Highlighted in survey of industry	Citations ^a	Efficacy				Epidemiological significance
				Chlorine ^b	Ozone	UV ^c	Filtration	
Protozoa	<i>Acanthamoeba</i>	No	ND	Low	Moderate	Unknown	High	Moderate
	<i>Cryptosporidium</i>	Yes	71	Low	High	High	High	High
	<i>Giardia</i>	Yes	50	Low	Moderate	High	High	Moderate
	<i>Naegleria</i>	No	ND ^e	Low	Moderate	Unknown	High	High
Phage	Bacteroidales phage	Yes	ND ^e	High	High	High	Low	None
	Coliphage	Yes	ND ^e	High	High	High	Low	None
Viruses	Enterovirus	Yes	23	Moderate	High	Low	Low	High
	Rotavirus	No	5	Moderate	Moderate	Low	Low	High
	Hepatitis A	No	4	Moderate	High	Low	Low	Moderate
	Noroviruses	No	1	Moderate	High	Low	Low	High
Bacteria	Coliforms	Yes	51	High	High	High	High	Low
	<i>Clostridium</i>	Yes	ND ^e	Moderate	Moderate	Moderate	High	Moderate
	Enterococci	Yes	ND ^f	High	High	High	High	Moderate
	<i>E. coli</i> (non-pathogenic)	Yes	42	High	High	High	High	Moderate
	<i>E. coli</i> (Pathogenic; e.g., O157)	No	NSD ^g	High	High	High	High	High
	<i>Salmonella</i>	Yes	10	High	High	High	High	High
	<i>Legionella</i>	Yes	10	Moderate	Moderate	High	High	High
	<i>Mycobacterium</i>	No	4	Moderate	High	High	High	Moderate
	<i>Shigella</i>	Yes	3	High	High	High	High	High
<i>Helicobacter</i>	No	3	High	Low	High	High	Moderate	

Legionella

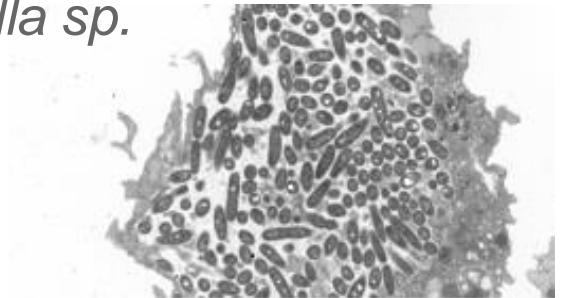
- Aerobic, non-encapsulated, non-spore forming, Gram-negative bacterium
- Consists of approximately 50 species consisting of 70 serogroups
- *L. pneumophila* has at least 35 serotypes
- Requires cysteine and iron for growth



ASM MicroLibrary.org © Delisle and Tomalty

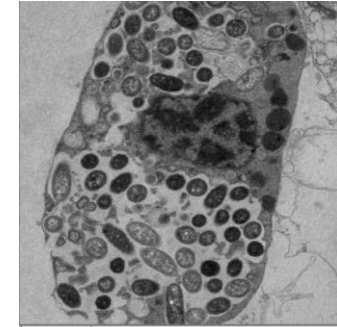
Legionella Ecology

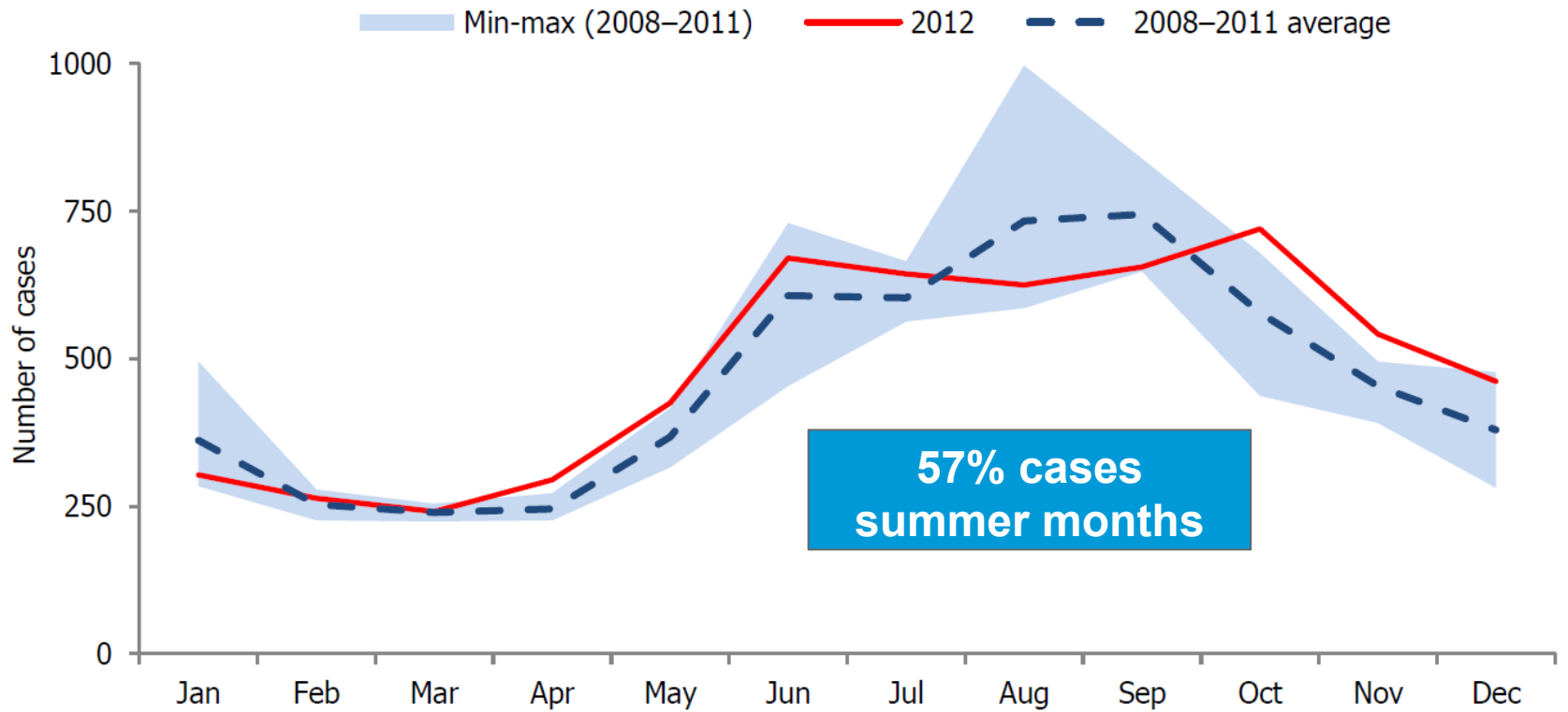
- Widely found in diverse water environments - groundwaters, drinking water systems, seawater and reclaimed water systems
- Engineered systems – hospitals, spas, cooling towers, humidifiers, ice machines, vegetable misters, cutting oils, etc.
- grow best at temperatures from 20°C to 42°C
- Acid tolerant and thermotolerant- surviving 50°C for several hours and tolerate temperatures up to 66°C
- Infect free-living amoebae such as *Hartmanella sp.* and *Acanthamoeba castellanii*.



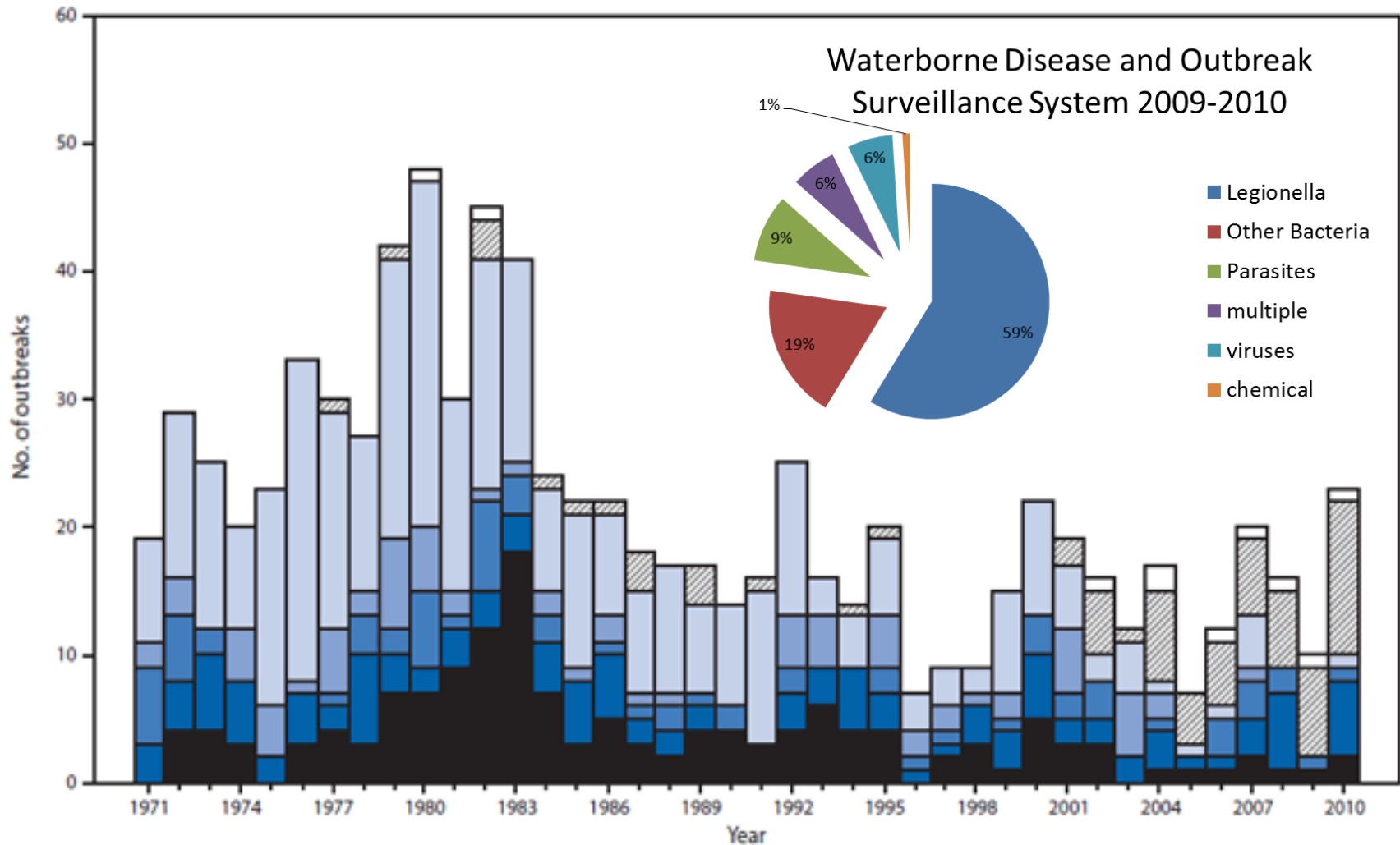
Legionellosis

- Legionnaire's disease - acute, sometimes fatal, pneumonia-infection. Nationally notifiable.
- Pontiac fever - self limiting mild illness
- *L. pneumophila* serogroup 1 is responsible for about 95% of the Legionnaires' Disease cases
- Causes 8,000 - 18,000 infections/year. Infections peak during summer months. About 4-5% of all pneumonia. Mortality 5-30%
- At risk individuals are >50 years age, immunocompromised, smokers, or those with chronic lung disease
- Infection acquired through inhalation
- In the lungs, the organism is engulfed by alveolar macrophages where they multiply and grow





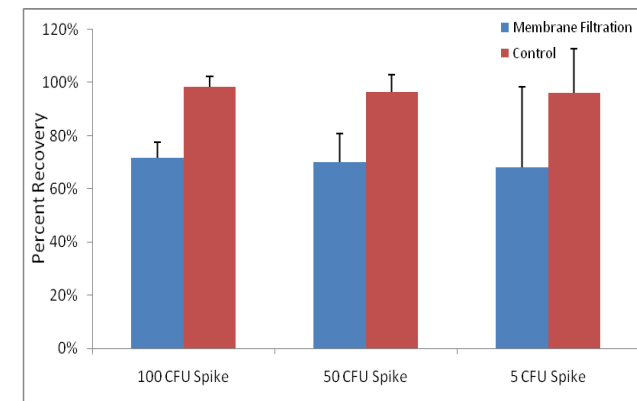
Drinking Water Disease Outbreaks, 1971–2010



Legionella Culture Method



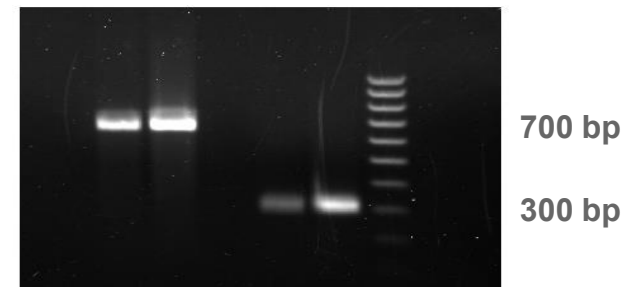
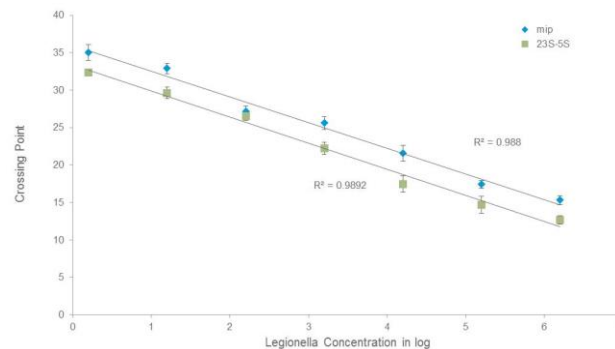
- 100 mL filtered through 0.45 μm pore size
- Filter placed in 10 mL phosphate-buffered solution
- Vortexed for 30 seconds
- 1mL mixed with an equal amount of acid (HCl-KCl, pH= 2.2 for 15 minutes, then neutralized with KOH-KCl.
- 0.1 mL spread-plated on BCYE agar supplemented with L-cysteine
- Incubated at 36.5° C under 2.5% CO₂ with 91% relative humidity for up to 10 days
- Typical colony is gray-white with a textured, cut-glass appearance
- Verification by latex agglutination tests to determine serotype



Legionella PCR Methods

- Evaluated two targets:
 - Macrophage infectivity potentiator (*mip*) gene
 - 23S-5S rRNA gene spacer region

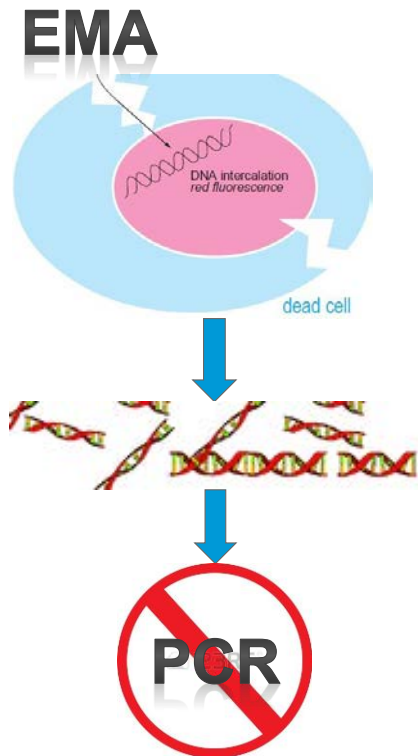
- PCR Sensitivity



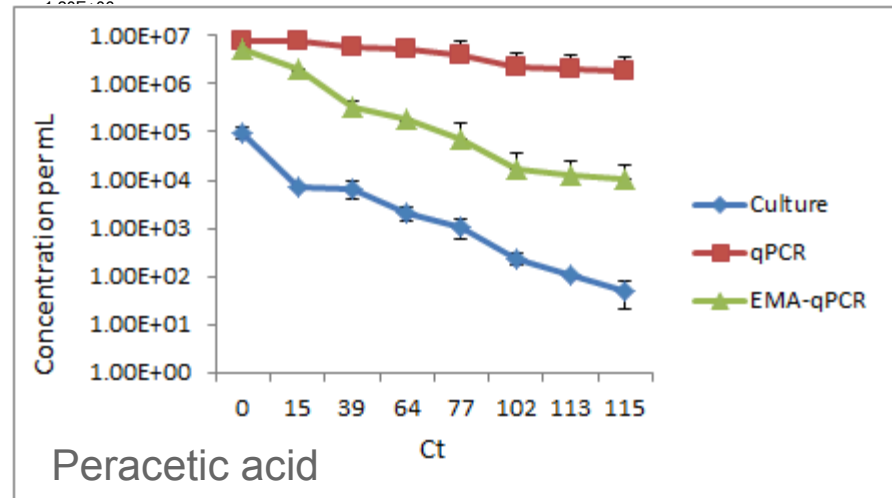
- PCR Specificity

- 99-100% identity with *Legionella pneumophila* (Philadelphia-1 strain, ATCC 33152),

Viable *Legionella* w/ ethidium monoazide



Allows PCR to detect only viable cells

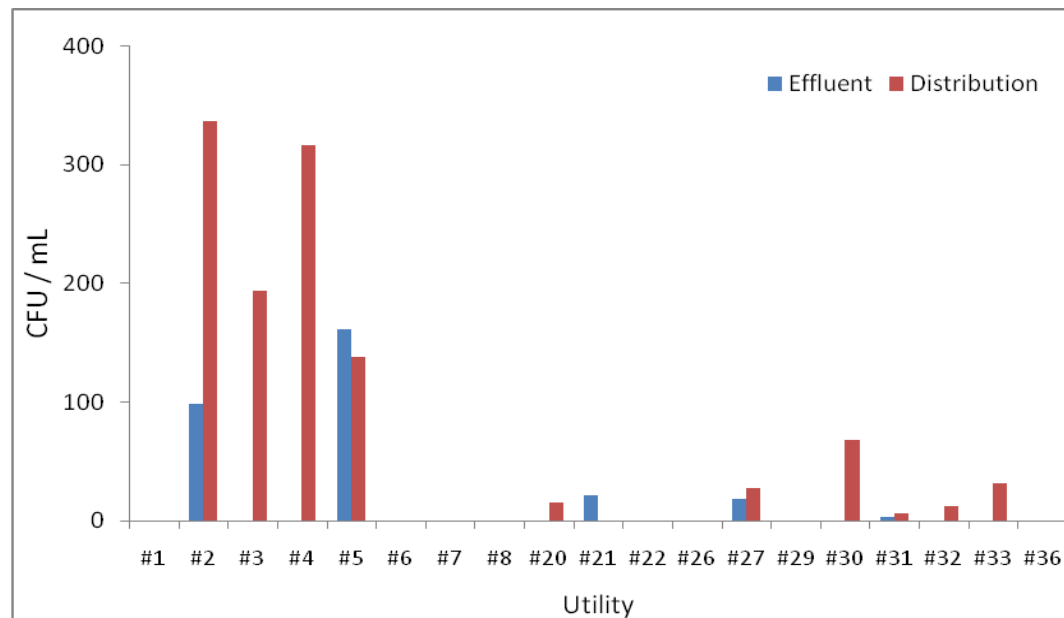
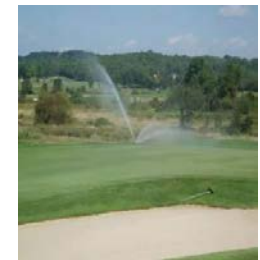


Utility	Concentration GU/mL		% Viable
	qPCR	EMA-qPCR	
TX - #27	45	18	40
FL - #30	28	11	39
CA - #4	339	125	37
FL - #31	517	120	23
CA - #32	617	56	9
AZ - #33	8	4	50

Legionella in Reclaimed Water

Characteristics of reclaimed water - growth/risk of *Legionella*

- Warm water
- High level of AOC
- No/little disinfectant residual
- Little distribution system cleaning/ flushing/ maintenance
- Spray irrigation

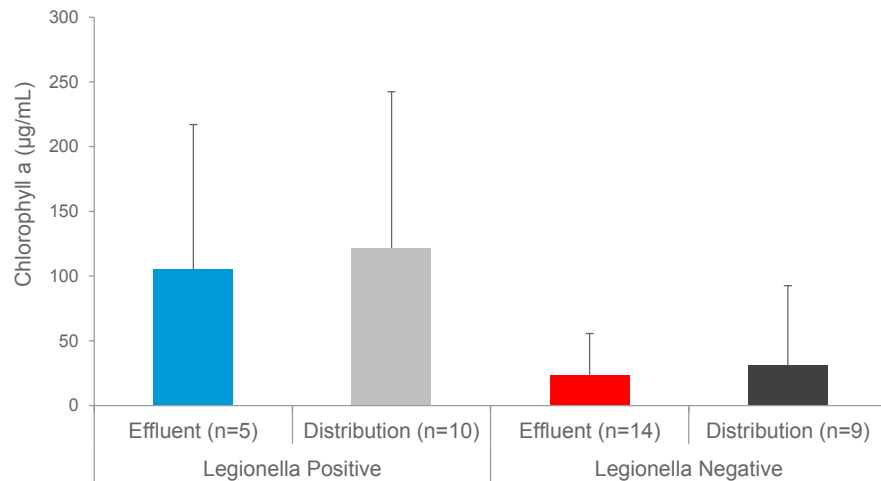


- 11 of 19 (58%) utilities positive
- 15 of 38 (39%) samples positive
- 5 effluents & 10 DS positive
- 115 CFU/mL in DS, 60 CFU/mL in effluent

Physical	Chemical	Operational	Biological
Temperature (°C)	pH	Design	Total coliform (cfu/100mL)
Corrosivity (mpy)	Conductivity	Flow rate (gpm)	<i>Legionella</i> (cfu/100mL)
Dissolved oxygen (mg/L)	Nitrogen (NO ₂ -N, NO ₃ -N, NH ₃ -N; mg/L)	Hydraulic pressure (psi)	<i>E. coli</i> (cfu/100mL)
Total suspended solids (mg/L)	AOC (mg/L)	Detention time (days)	Heterotrophic bacteria (HPC/mL)
Turbidity (NTU)	BOD (mg/L)	Flushing	Algae
	COD (mg/L)	Reservoir management	
	TDS (mg/L)	Aeration - mixing	
	Total chlorine (mg/L)	Short circuiting	
	Free chlorine (mg/L)	Run-off control	
	Phosphorus (mg/L)	Cleaning and dredging	
	Salinity (mg/L)	Distribution system operations	
	DPBs (HAA, TTHM; µg/L)		
	TOC (mg/L)		
	H ₂ S (mg/L)		
	Fe ³⁺ (mg/L)		
	Mn ²⁺ (mg/L)		

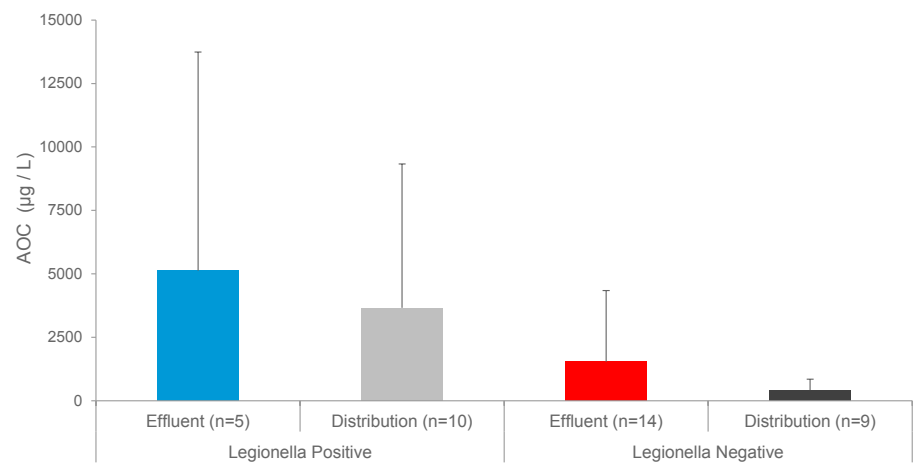
Chlorophyll

R² 0.625



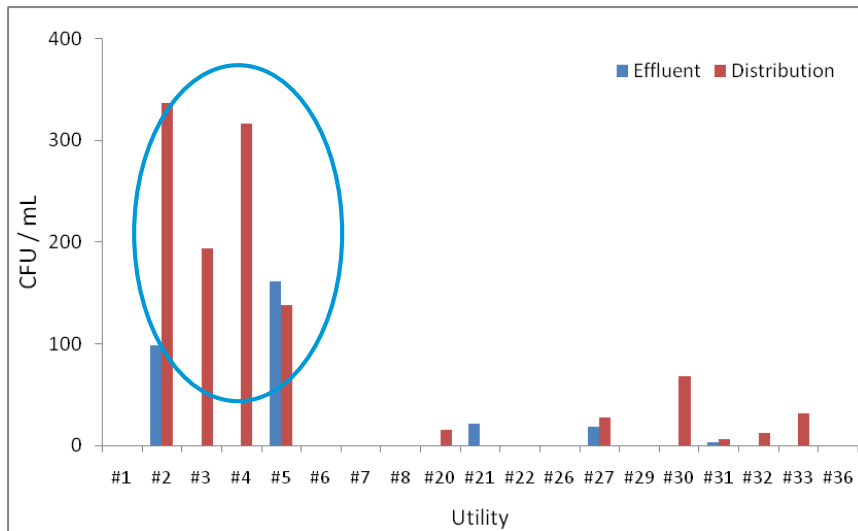
AOC

R² 0.385

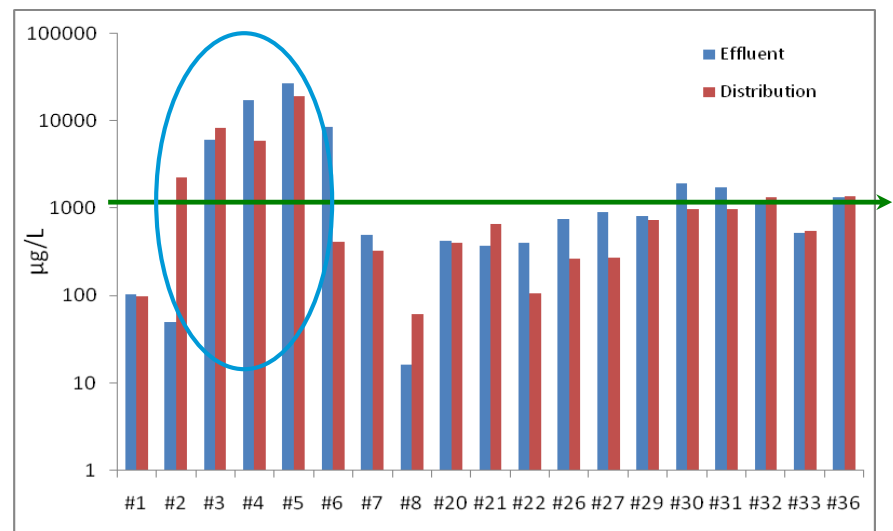


Evidence of Regrowth

Legionella

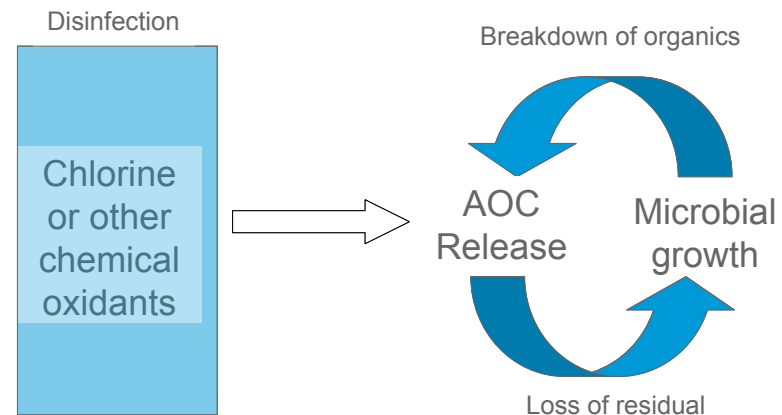
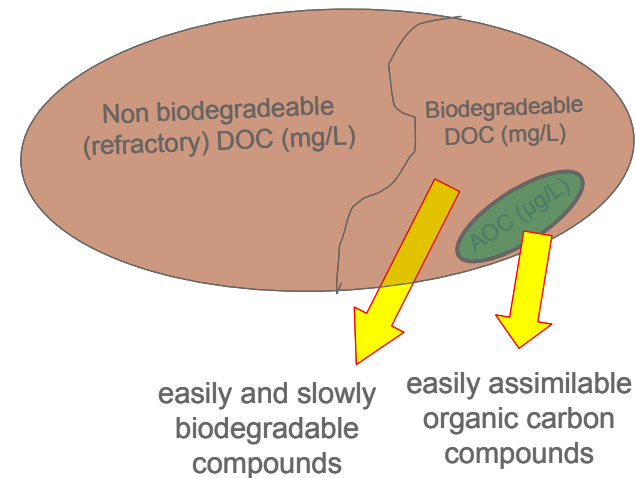


AOC levels



Microbial Regrowth

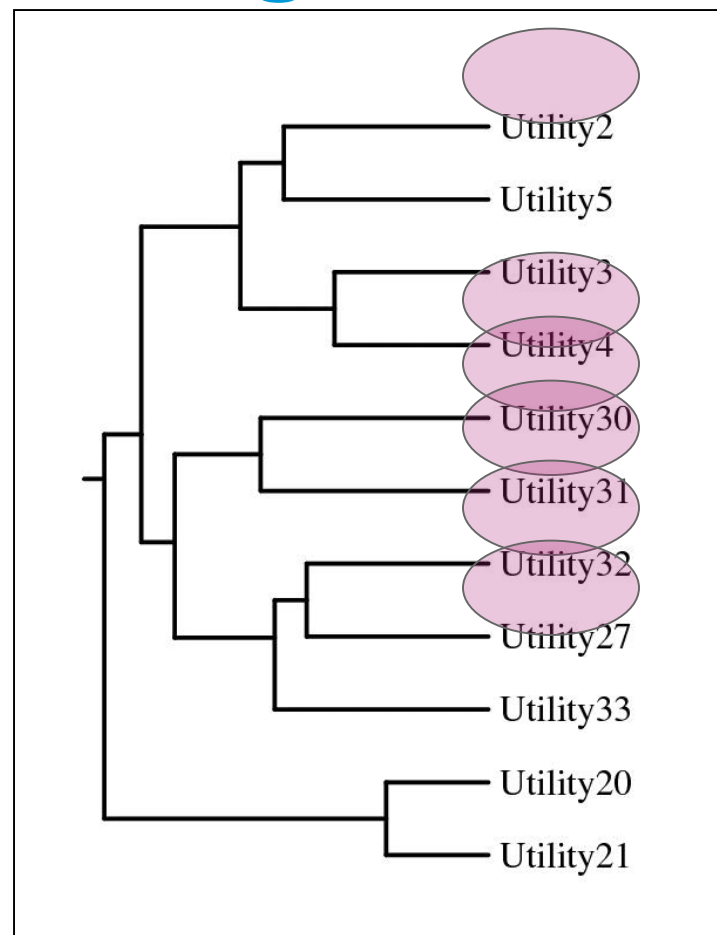
- Low disinfectant
- Warm temperatures
- Abundant food source
(i.e. DOC, BDOC, AOC)
- Coagulation, sedimentation, filtration reduce BDOC
- Chemical disinfection increases biodegradable compounds



Selection of Systems for Extensive Monitoring

Selected systems based on:

- *Legionella* occurrence
- Production capacity
- Treatment technology
- Disinfectant,
- Storage type etc



Detection of *Legionella*

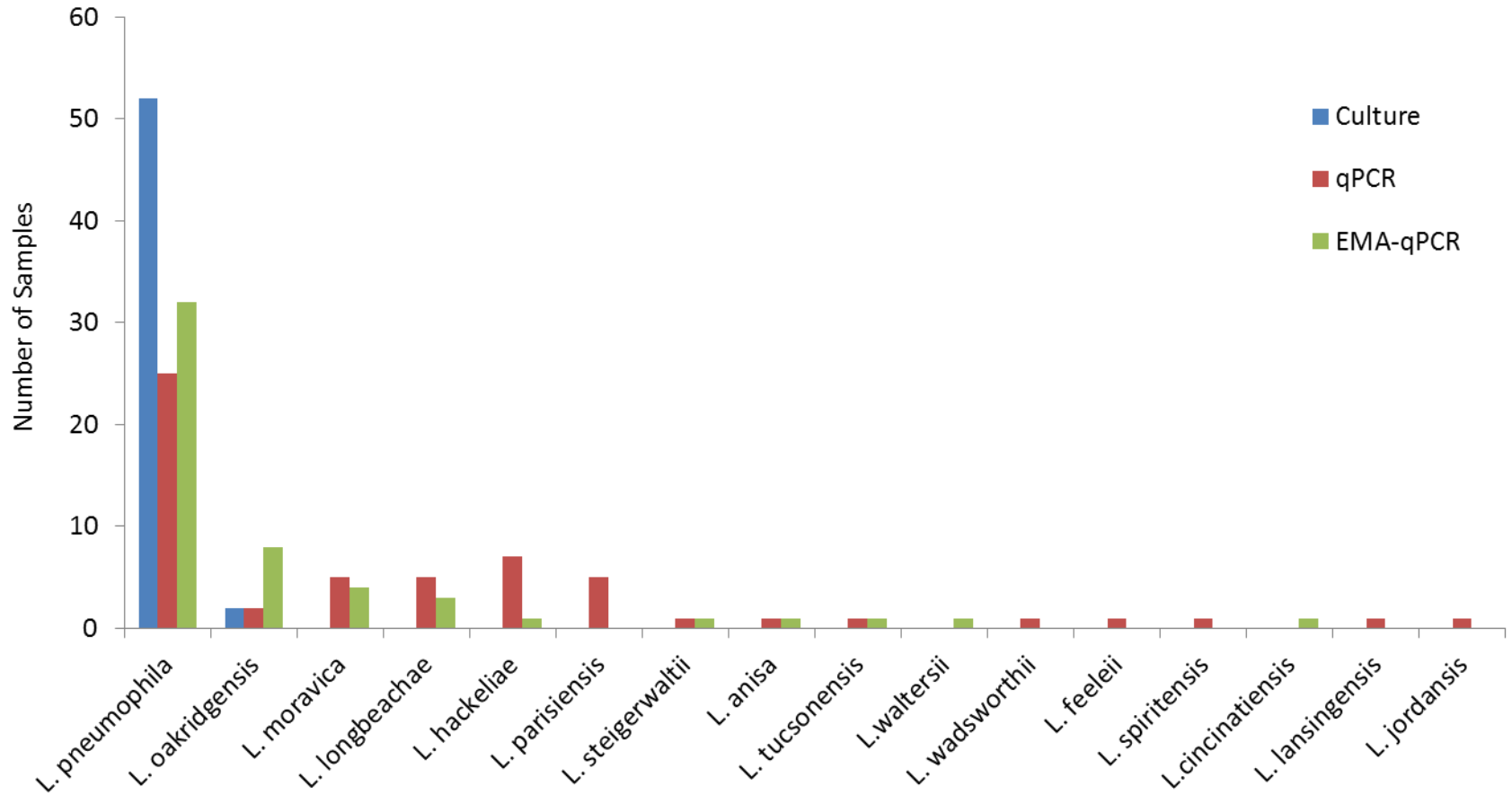
Utility	% Occurrence			Concentration /mL		
	qPCR	EMA-qPCR	Culture	qPCR (GU)	EMA-qPCR (GU)	Culture (CFU)
TX - #27	79	74	47	45	18	3
FL - #30	55	55	35	28	11	2
CA - #4	100	100	81	339	125	11
FL - #31	100	100	75	517	120	10
CA - #32	100	100	60	617	56	4
AZ - #33	45	45	5	8	4	0.2

N = 20 samples, March to December, 2014

Water Quality Summary

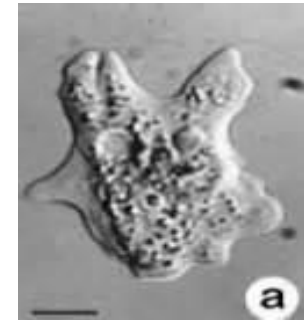
Utility	AOC (µg/L)	TOC (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)	Phosphorus (mg/L)	Total Chlorine (mg/L)	Free Chlorine (mg/L)	Turbidity (NTU)	pH	Water Temp. (° C)
TX- #27	662	7.6	19.6	0.4	0.2	15.9	0.4	0.1	3.6	7.1	24.0
FL - #30	1629	15.8	2.0	0.7	0.5	5.1	0.2	0.1	6.2	7.7	25.6
CA - #4	1564	10.7	2.5	4.7	0.8	2.3	1.1	0.1	11.4	8.1	21.7
FL - #31	1402	17.0	1.4	15.0	3.0	1.5	1.2	0.1	1.4	7.1	24.9
CA - #32	1079	10.2	1.3	33.6	0.4	1.8	3.0	0.1	1.9	6.9	27.7
AZ - #33	505	4.7	2.7	0.3	0.0	4.7	0.5	0.3	0.6	7.7	28.7

Legionella species identified by method



Legionella and Amoebae

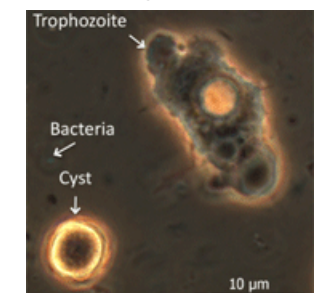
- Intracellular *Legionella* in: *Acanthamoeba*, *Amoeba*, *Comandonia*, *Echinamoeba*, *Filamoeba*, *Hartmannella*, *Naegleria*, *Paratetramitus*, *Vahlkamfia*, *Tetrahymena*, *Dictyostelium*
- *Legionella* survive for months, resistant to 50 mg/L free chlorine for 18 hr
- Coated with amoebal proteins
- Increases virulence, replication
- *Legionella*-containing vacuoles expelled prior to encystation
- Trophozoite stage sensitive to disinfectants ($CT_{99.9} = 1.5 \text{ mg-min/L}$)



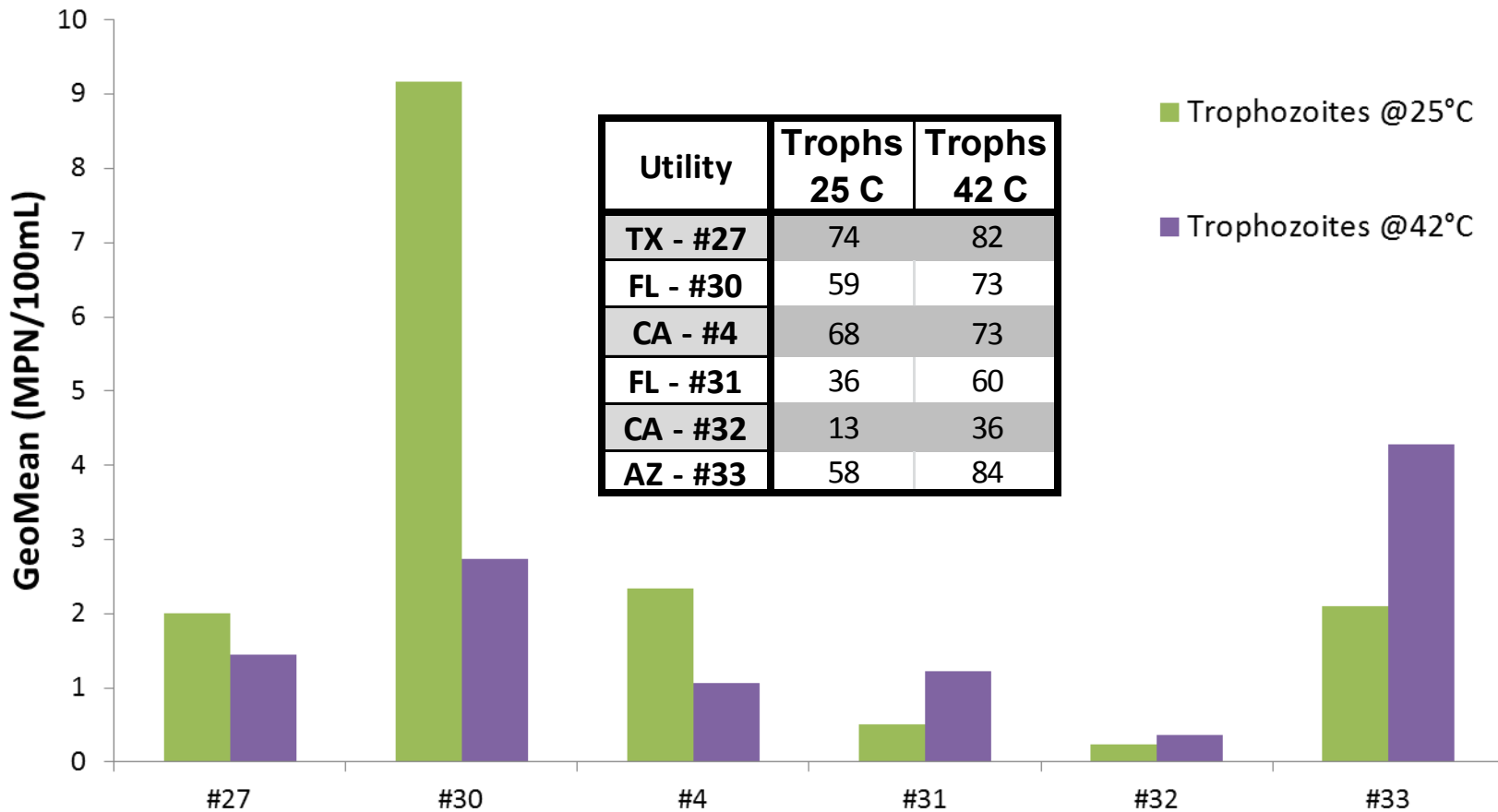
Trophozoite



Cyst

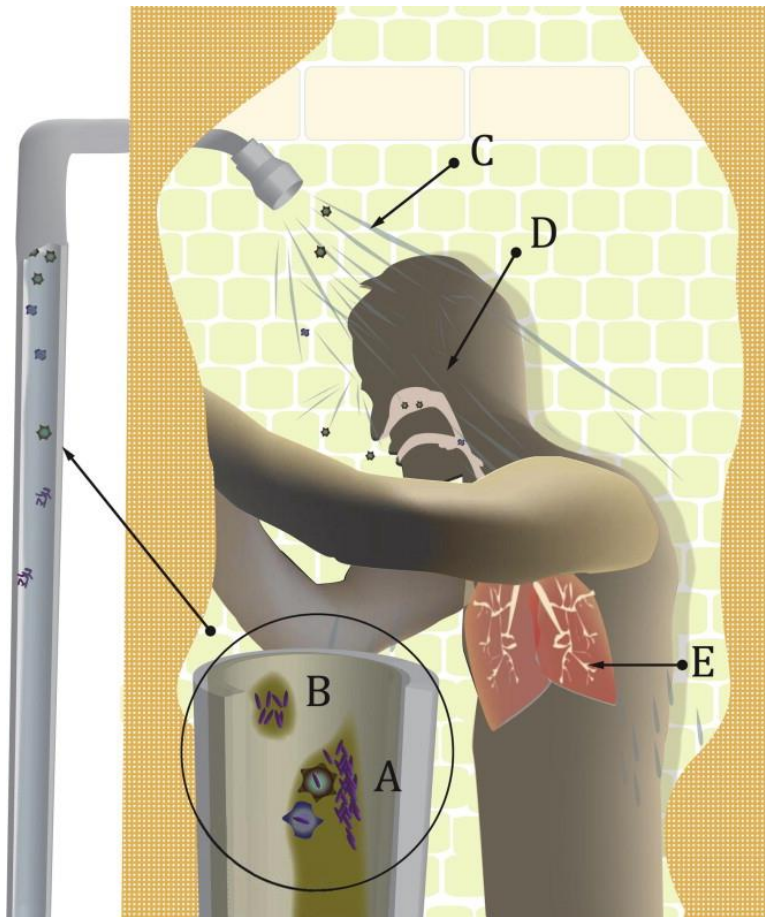


Trophozoite Concentration



Chloramines

Conceptual Risk Model



- A. *Legionella* in biofilm or amoebae
- B. Detachment
- C. Aerosolized
- D. Inhaled
- E. Deposited in lungs

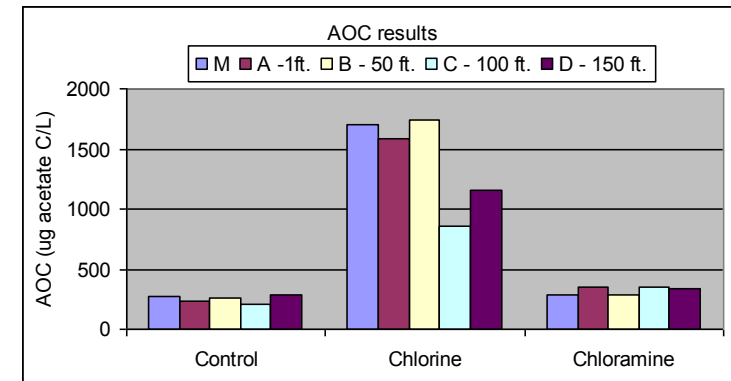
Critical range: 3.5×10^3 - 3.5×10^5 cfu/mL

Managing *Legionella* Risk

- **Determining *Legionella* densities, species and serotypes**
 - Methods for culture and PCR

- **Emphasis on maintaining a disinfectant residual**
 - Some advantage for a chloramine residual
 - More persistent
 - Impact on encycystation
 - Investigation of other biocides
 - peracetic acid?

- **Distribution System BMPs**
 - recommendations from project WRRF-11-03



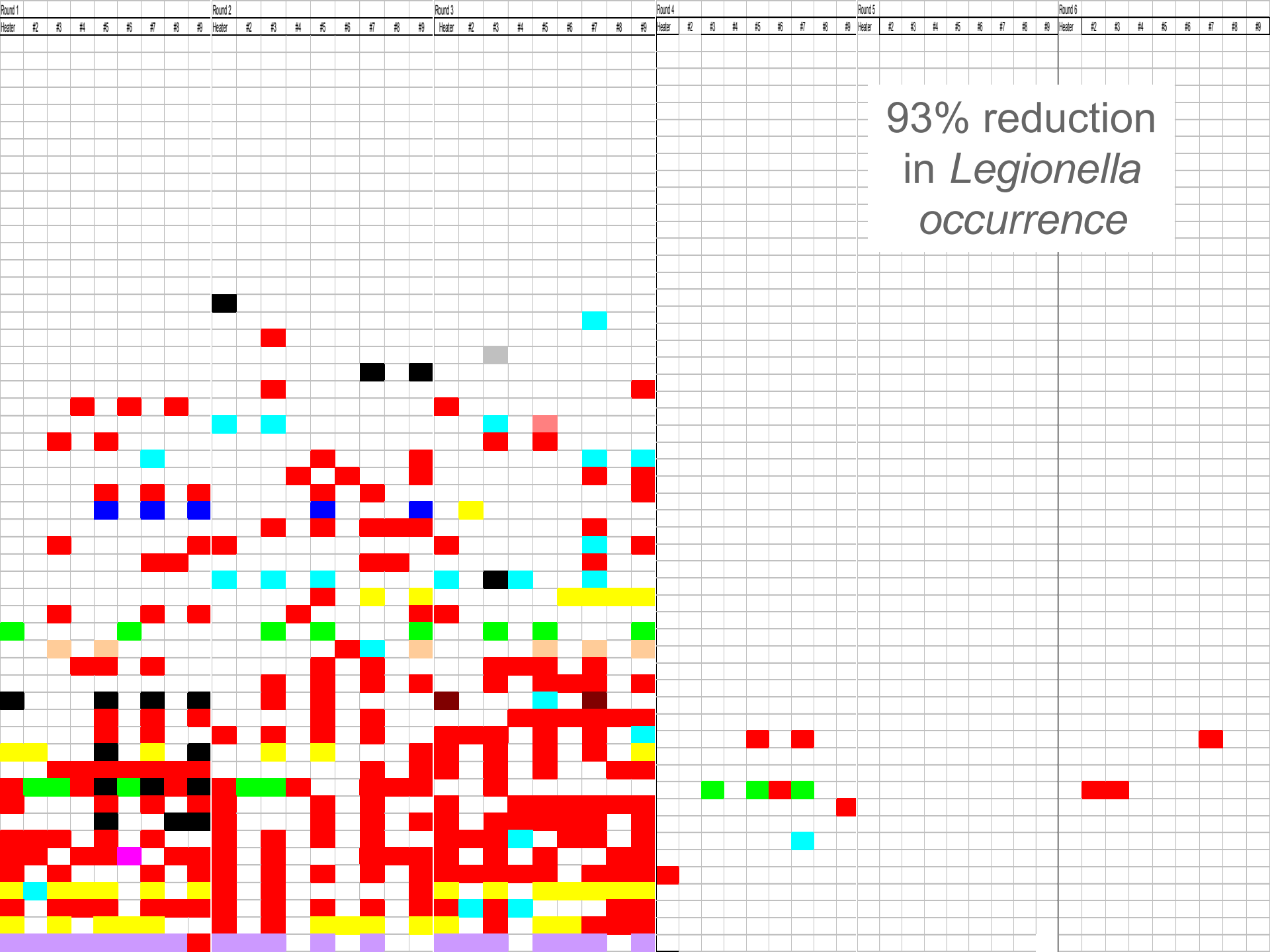
From Jjemba et. al. 2010, WRF – 05 – 002

Lessons from Real Life: San Francisco, CA

- 53 buildings
- Sampled 3 times pre- and post-conversion to chloramines
- Sampled hot water heater and four distal sites
- Sampled swab and water from distal sites
- Surveys collected data on building age, height, type and number of hot water heaters
- pH, temperature, free or total Cl₂ residual measured for each sample

Flannery, B. et al. 2006. Reducing *Legionella* colonization of water systems with monochloramine. Emerg. Infect. Dis. 12(4): 588-596.

<http://www.cdc.gov/ncidod/EID/vol12no04/05-1101.htm>.



93% reduction
in *Legionella*
occurrence

Summary

- ***Legionella* is an emerging public health concern for utilities**
- **There is a gap in information on the ecology of free living amoebae & currently risk models are incomplete**
 - Need faster, more accurate methods
- **Existing data demonstrates that *Legionella* spp. are present in water distribution systems and premise plumbing**
 - *Legionella* association with amoeba increases numbers, occurrence, virulence and resistance to chlorination
- **Evidence to support chloramination better than strong oxidants for *Legionella* control**
 - Focus on maintaining a disinfectant residual, regular flushing/cleaning

WE CARE ABOUT WATER. IT'S WHAT WE DO.

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