Pathogen/biofilm studies to aid QMRA & research planning

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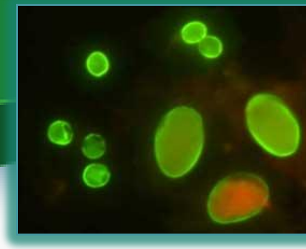
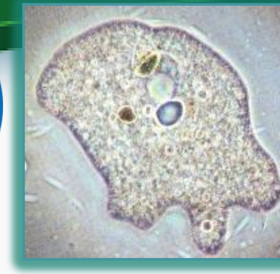
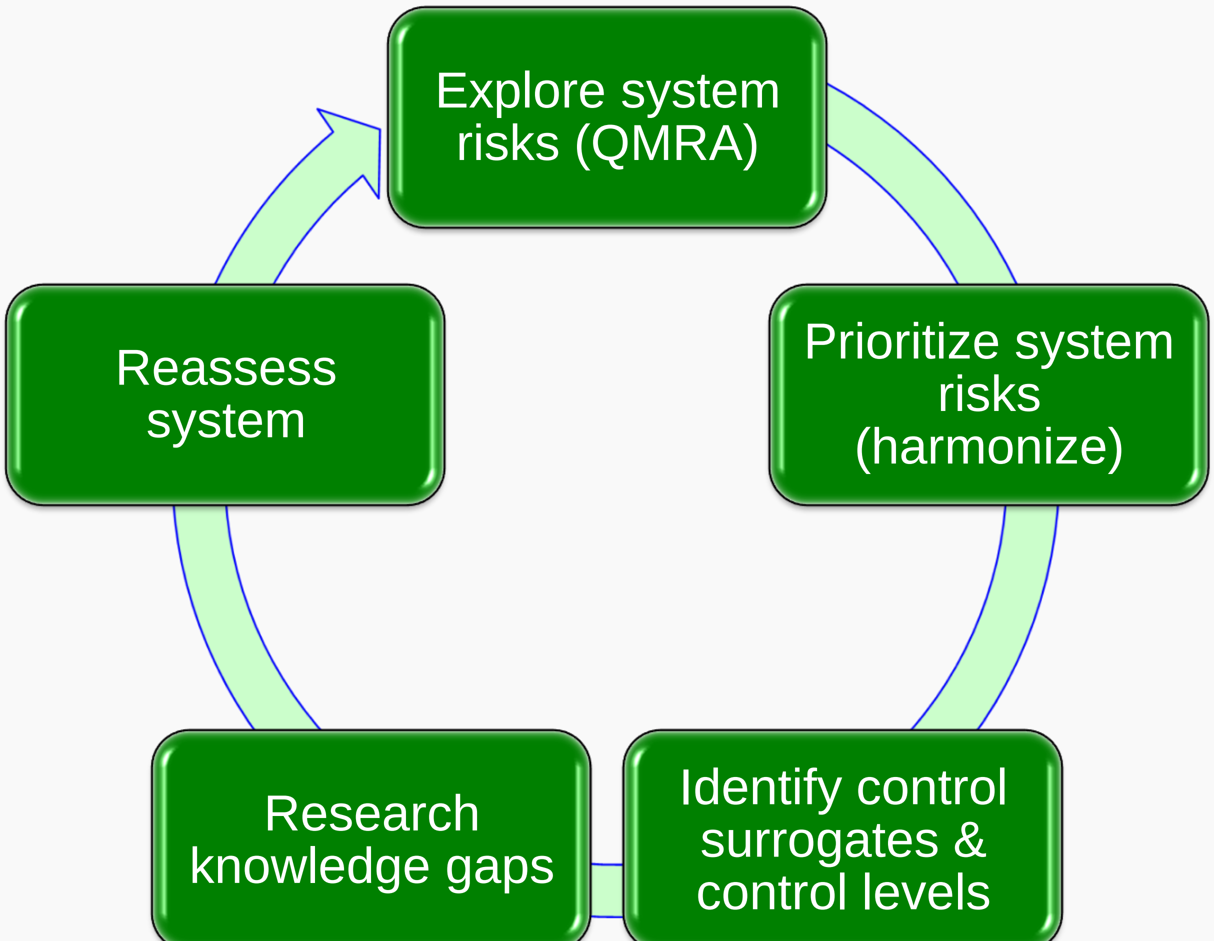



QMRA – Analytic Framework



Quantitative microbial risk assessment (QMRA)

STEP 1
SETTING

Problem formulation & Hazard identification
Describe physical system, selection of reference pathogens and identification of hazardous events

STEP 2
EXPOSURE

Source water
Pathogen concentration

Treatment
Pathogen removal

Ingress/Growth
Enteric/Environmental pathogens

($P_{ingress}$)

Distribution
Pathogen fate (in biofilm/free)

Consumption
Volume water consumed

STEP 3
HEALTH EFFECTS

Dose-Response (P_{inf})
Selection of appropriate models for each pathogen and the population exposed

STEP 4
RISK

Risk Characterisation
Simulations for each pathogen baseline and event infection risks with variability & uncertainty identified

STEP 1

SETTING

Hazard identification & characterization
Describe physical system, selection of reference pathogens and identification of hazardous events

AF-RICP issues 2010

STEP 2

EXPOSURE

For each reference pathogen:

Ingress of enterics (via pressure transients, main repairs etc.)

$(P_{ingress})$ →

Source water
Pathogen density (PDF)

↓

Treatment
Pathogen removal

↓

Distribution
Pathogen loss (biofilm/death)

↓

Consumption
Volume water inhaled/ingested

DWDS *Norovirus* risk*

- Maintaining a free chlorine residual of 0.2 mg/L or above is the last defense against the risk of viral infection due to negative pressure transients
- Maintaining a chloramine residual did not appear to significantly reduce viral risk
- Effectiveness of ensuring separation distances from sewer mains to reduce risk may be system-specific
- Leak detection/repair and cross-connection control should be prioritized in areas vulnerable to negative pressure transients

*Yang *et al.* (2011) J Water Health **9:**291–305

GI RR 1.6 if low pressure Nygård *et al* (2007) Int J Epi 36:873-880

Public health costs from water

- CDC estimate waterborne disease costs > $970 m/y
  - Addressing giardiasis, cryptosporidiosis, Legionnaires' disease, *otitis externa*, and non-tuberculous mycobacterial infections, causing over 40 000 hospitalizations per year

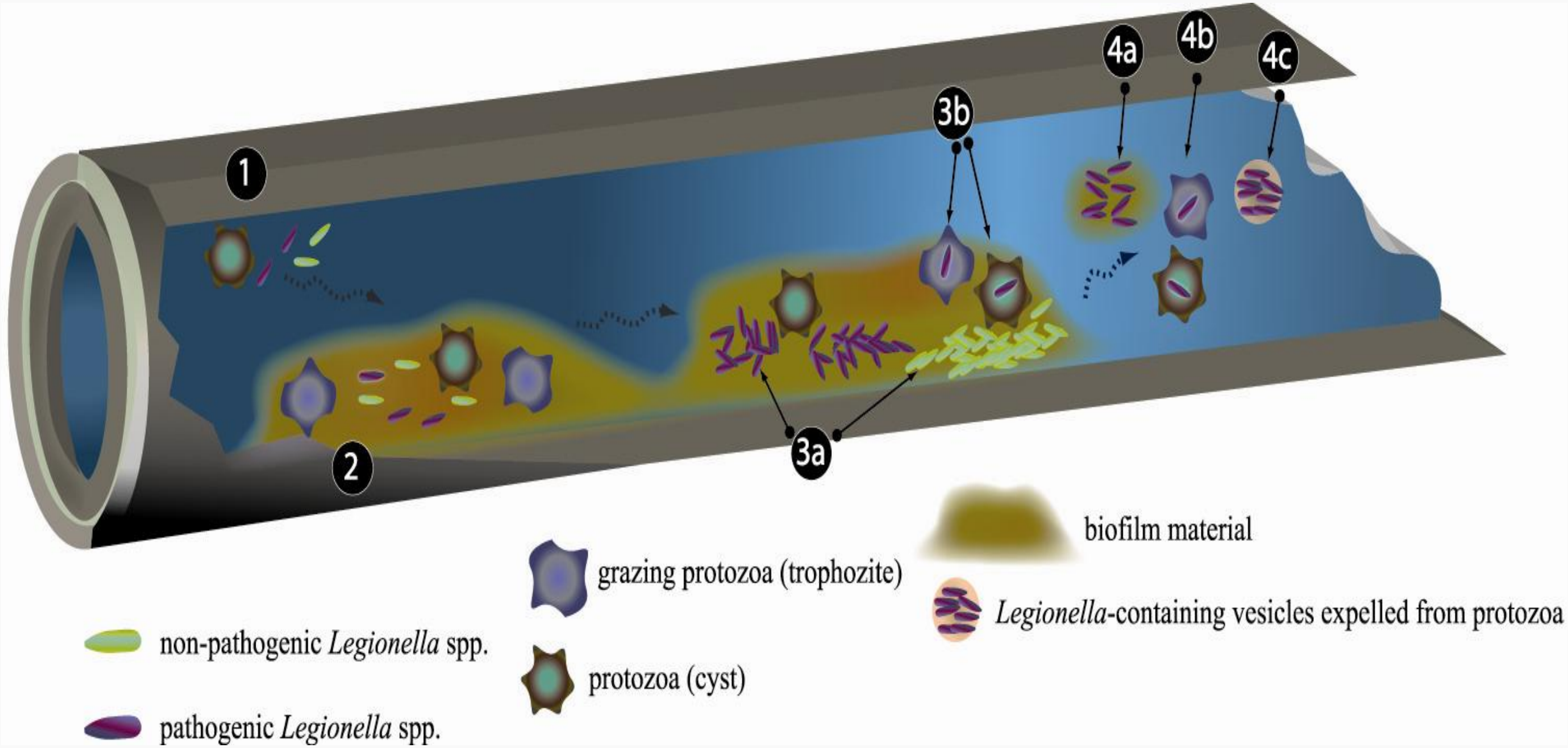
Disease	**$ / hospitalization**	**Total cost**
Cryptosporidiosis	**$16 797**	**$45 770 572**
Giardiasis	**$9 607**	**$34 401 449**
Legionnaires' disease	**$33 366**	**$433 752 020**
NTM infection/Pulmonary	**$25 985 / $25 409**	**$425 788 469/ $194 597 422**

Collier *et al.* (2012) Epi Inf 140(11), 2003-2013

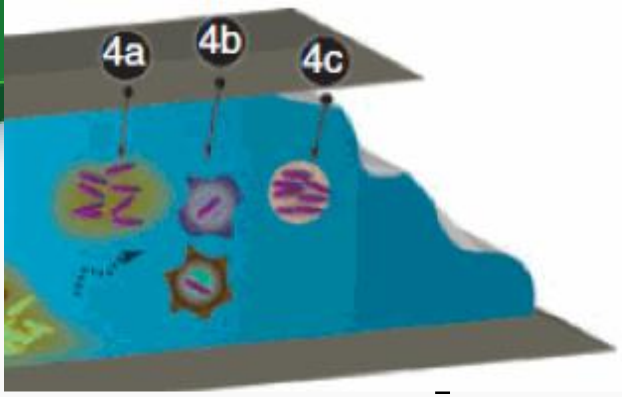
Also, nutrients & environmental pathogens even more likely intrude than enteric pathogens

- Providing continued input of environmental pathogens to storage reservoir sediments, pipe biofilms and in-premise pipe surfaces
  - i.e. for growth of NTM, legionellae, *Pseudomonas aeruginosa* etc. if other ecological conditions allow

Conceptual *Legionella* model: piped water



Lau & Ashbolt (2009) J Appl Microbiol 107(3):368–378



Legionella biofilm release scenarios

Risk size: < 7 micron aerosols

a) *Legionella* that have proliferated within **biofilm** released as this material sloughs off

b) Within released amoebae **trophozoites**

c) Within **vesicles** excreted by amoebae or

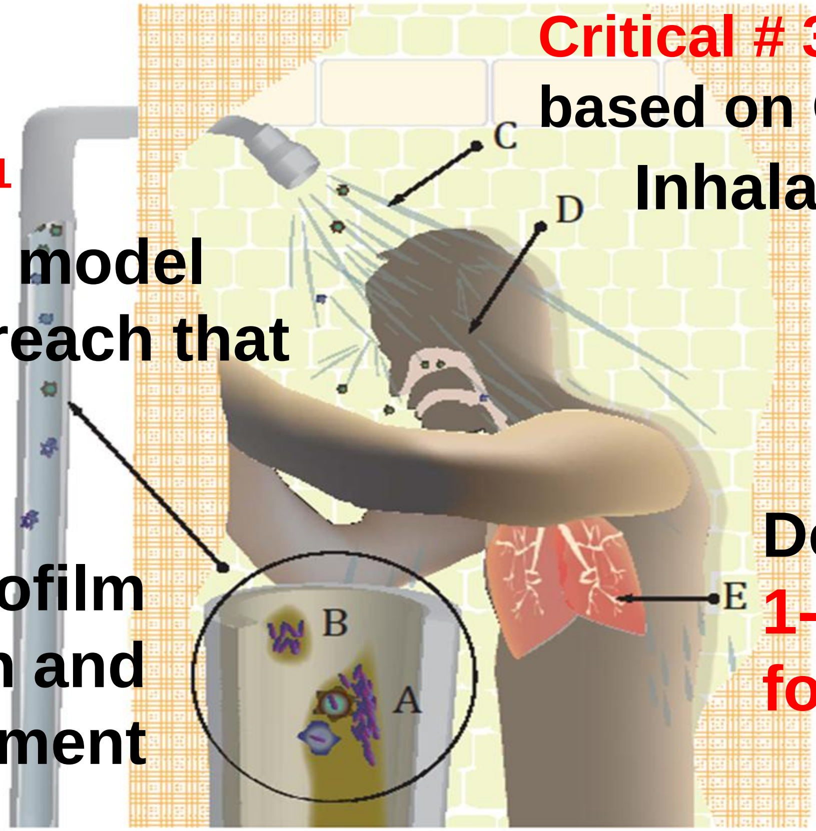
d) Within released amoebae **cysts**

QMRA for critical *Legionella* densities

Critical # in DW
10^6 – 10^8 CFU L^{-1}
based on QMRA model
Needs hosts to reach that

Aerosolization
Critical # 35 – 3,500 CFU m^{-3}
based on QMRA model

Inhalation



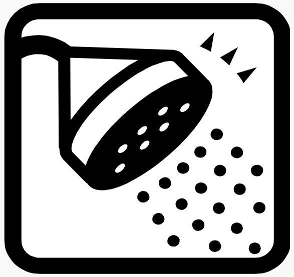
Biofilm colonization and detachment

Deposition
1-1,000 CFU in lung for potential illness

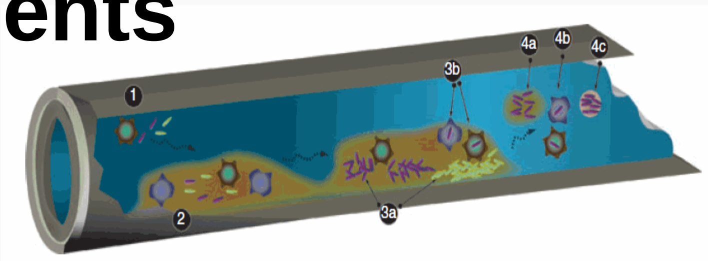
Schoen & Ashbolt (2011) Water Research 45(18): 5826-5836

Environ Path research gaps (2010)

- Partitioning coefficients needed for a variety of environmental pathogens



- Fraction of community-acquired respiratory disease from *Legionella*, MAC/NTM?
- **Host-pathogen ecology-management in water system environments**

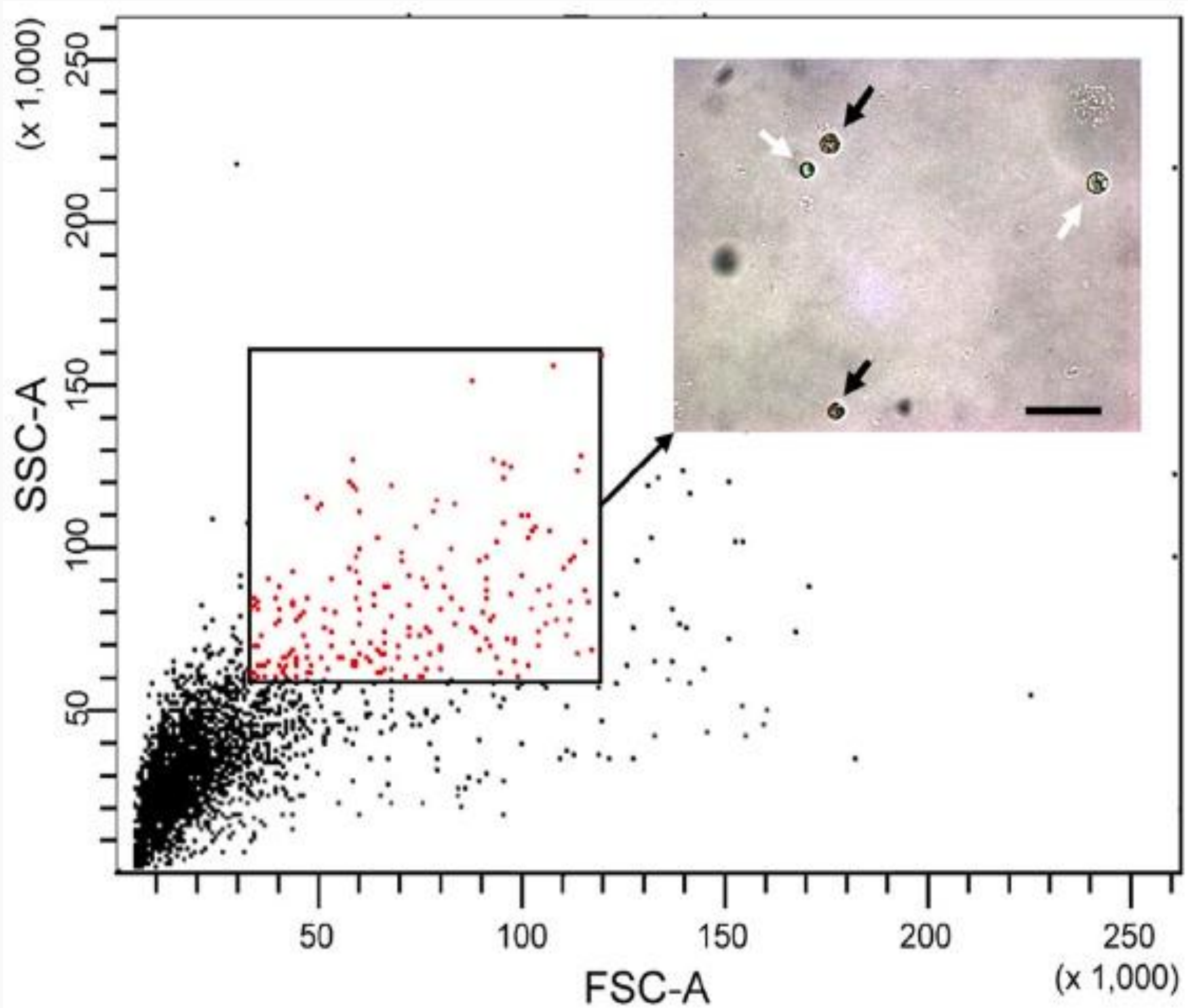


How many *Legionella* per amoeba?

Flow cytometer sorted (red zone) & culture to estimate *Legionella* CFU trophozoite^{-1}

- *Acanthamoeba polyphaga* 1,348 (mean, 329)
- *Naegleria fowleri* 385 (mean, 44)

Buse & Ashbolt (2012) AEM 78(6):2070-2072



Drinking water biofilm microbiota

- Metagenomics undertaken to date shows
  - 18S rDNA (amoebae, nematodes, rotifers), e.g.
    - amoebae: ***Hartmannella vermiformis***, & species of *Echinamoeba, Pseudoparamoeba*, *Protacanthamoeba*, *Platyamoeba & Vannella*

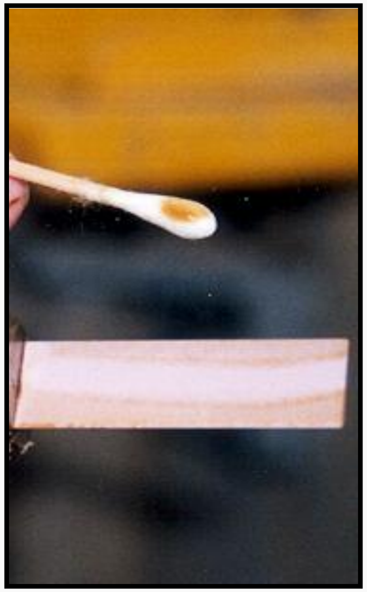
Buse *et al.* (accepted) Env Sci Pollut Res

  - 16S rDNA (*Bacteria*) for NH_2Cl-treated water
    - After 3-4 months 'stable' community of *Actinobacteria*, *Bacteroidetes* & *Proteobacteria,* with **>30% *Mycobacterium* spp.**

Revetta *et al.* (accepted) FEMS Micro Lett

Current *Legionella* disinfection/control

- Thermal (80 °C, 10 min)
  - most effective, but only if repeated frequently (used in health care centers)
- Monochloramine (NH_2Cl) more effective than free chlorine (HOCl)
  - planktonic
  - sessile/biofilm (increased resistance)
  - amoeba-bound (further increased resistance)



- Point of use control: Filtering & Cu/Ag ions used in hospital/care facilities (impact on culturable cells)

(Storey *et al*. 2004 *Scand J. Infect Dis* **36**: 656-662)

Does aged biofilm on copper pipe suppress *Legionella* vs on PVC?

- Spiked 1 y-old PVC & Cu lab DW pipe biofilms, followed 4 mo
- *L. pneumophila* maintained cultivability in PVC biofilms compared to below detectable CFUs from Cu biofilms
- However, *L. pneumophila* cells shed in reactor effluent water reflected persistent VBNC *L. pneumophila* (more if + amoebae) within Cu-coupon vs few VBNC with PVC-coupon reactors
- Also effluent samples from inoculated Cu reactors contained more & for months longer culturable *L. pneumophila* than PVC inoculated reactors

Buse *et al.* (accepted) Int J Hyg Environ Health

Stress & *Legionella*'s transcriptome

- **Methods**: CuO-nanoparticle exposure as the stressor for *L. pneumophila* Philadelphia 1:
  - Using a whole genome *Legionella* microarray
  - RT-qPCR assays of expressed mRNA
- Identified expression of genes involved in metabolism, transcription, translation, replication-repair, and virulence (e.g. *ceg29* and *rtxA*)
- Now using RT-qPCR to understand biofilm & intra-amoebal stress for *Legionella* vs. various disinfectants

Lu *et al.* (2013) Appl Environ Microbiol 79: 2713-2720

Legionella QMRA summary

- Wide potential dose of concern in lungs
- Air-water partitioning drives the dose
- Critical *Legionella* densities likely high
  - So sig numbers readily detectable, and
  - Low numbers in drinking water minimal direct concern, but seed downstream premise plumbing
  - Various amoebae host common, reduc. disinfection
- Potential risk not associated with fecal indicators, & $NH_3Cl \rightarrow$ increased MAC risk

Pathogen-biofilm timeline

2013 Buse: 16/18S rDNA Cu/PVC

2012 Lu: *Legionella* transcriptome

2011 Buse: *Leg* counts in amoebae

2011 Schoen & Ashbolt QMRA shower *Leg.*

2011 Thomas & Ashbolt: *Leg.* risk recycled water

2009 Lau & Ashbolt: *Legionella* biofilm model

2009 Valster: DW 18S rRNA gene sequence amoebae ID

2008 Falkinham: *Mycobacterium avium* shower pulmonary disease

2007 Cooper: *E. coli* O157 growth environ biofilms, Juhna: VBNC DW pipes

2005 Donlon: *Legionella* ecology within biofilm amoebae
Schuster: first pyrosequencing of environmental bacteria
2010 Hong: first pyrosequencing of drinking water biofilms

Conclusions: QMRA gaps

- What fraction of qPCR positives are viable or infectious, by water disinfection process
- What fraction of pathogens release from filters & biofilms and under what conditions
- Need to correlate qPCR targets to actual pathogens for different environments (F & T)
- Need dose-response data for environmental pathogens and enteric viruses

Disclaimer/Acknowledgements

The views expressed in this presentation are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency

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