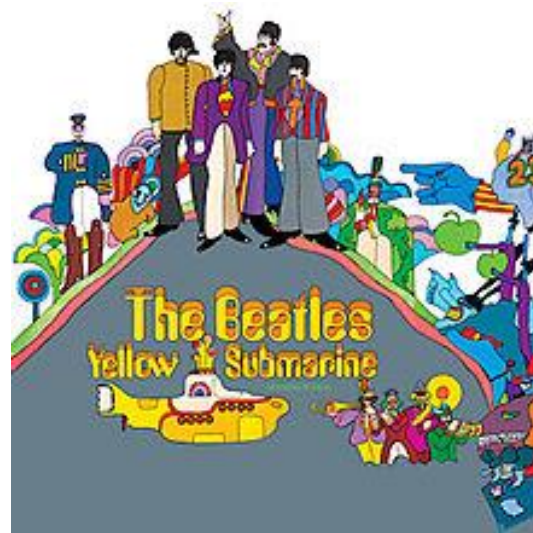




# Legionellosis



## WCLN Regional Meetings September 2019

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# Acknowledgement

- Anna Kocharian
  - Epidemiologist, Communicable Diseases  
Epidemiology Section, WI Department of Health  
Services



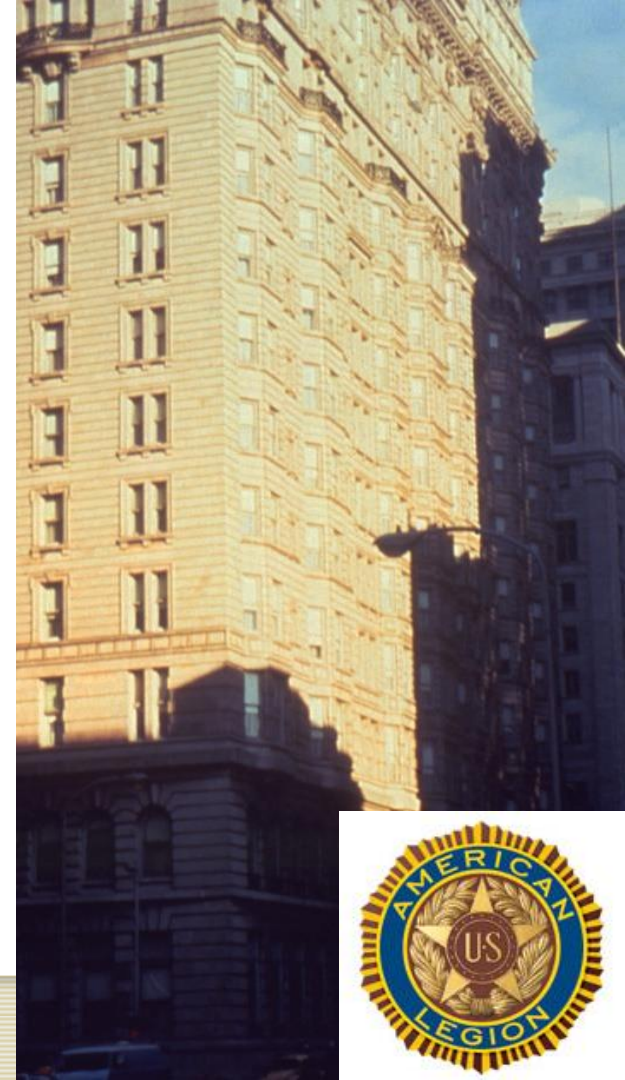
# Outline

- Microbiology
  - Organism and clinical aspects
- Transmission
- Big-picture epidemiology
- WI DPH memo
- Outbreak example



# History of Legionnaires' Disease

- American Legion Convention, Philadelphia 1976
- Epidemic pneumonia, 15% fatality rate
- Joseph McDade, others at CDC determined novel Gram-negative bacteria was the cause
- Bacteria named *Legionella pneumophila*





# *Legionella* species

- 59 validly named species
- *L. pneumophila*, *L. micdadei*,  
*L. longbeachae*, *L. dumoffii*  
most important clinically
  - *L. pneumophila* causes >90% of Legionnaires' disease
- Closest relatives are *Coxiella*
  - Share intracellular parasitism and close homologies with virulence genes





# *Legionella* species

- Almost all of the 59 species have been isolated from aqueous environmental sources
  - A third isolated from both humans and environment
  - Humans are accidental hosts
- *L. longbeachae* mainly a soil organism
  - After exposure to soil, including potting soil
- Environmental *L. pneumoniae* is parasite of free-living amoebae
  - *Acanthamoeba*, *Naegleria*, etc.





# *Legionella* genus

- *Legionella* spp.
  - *Legionella micdadei*
  - *Legionella longbeachae*
  - ...
  - *Legionella pneumophila*
    - Serogroup 1
      - Pontiac MoAb subtype/subgroup
    - Serogroup 2
    - Serogroup 3
    - ...



# *Legionella*

- Community-acquired Legionnaires' disease:
  - *L. pneumophila* serogroup 1 causes 95-98%
    - Pontiac MoAb subgroup causes 80-90%
- Nosocomial Legionnaires' disease:
  - Up to 60% caused by:
    - other *L. pneumophila* 1 subtypes
    - other *L. pneumophila* serogroups
    - other *Legionella* spp.

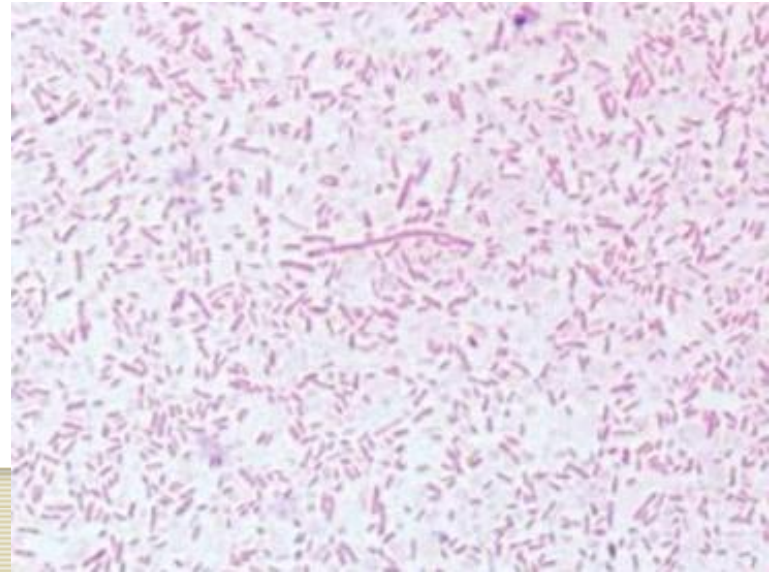






# *Legionella pneumophila* Gram Stain

- Morphology of *L. pneumophila*:
  - Lung and sputum: small coccobacillus to short rod (3-5 $\mu$ m)
  - Culture plate: long filamentous bacillus (10-25 $\mu$ m)
  - Difficult to detect by Gram staining sputum or lung biopsy specimens
    - 0.1% basic fuchsin, rather than safranin, enhances staining from culture plates
    - Even with fuchsin, difficult to visualize the bacterium in sputum and tissues





# Urinary antigen testing

- Easy and fast
- Most positive patients identified by UA
- Targeted toward the Pontiac subtype
- Sensitivity:
  - 90-95% with severe LP serogroup 1 with Pontiac subtype
  - 50% in outpatients with mild disease with Pontiac subtype
  - ~40% of hospitalized with other LP serogroup 1 serotypes
  - 5-40% of hospitalized with other LP serogroups and other *Legionella* spp.





# Clicker question 1

Does your lab perform *Legionella* urine antigen testing?

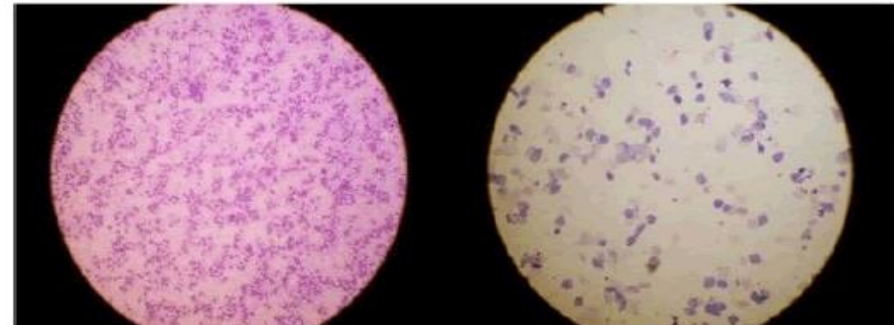
- A) Yes
- B) No
- C) I'll have to get back to you...



# Legionella Culture

- Sputum microscopic scoring criteria **cannot** be used to determine which sputum specimens to culture for *Legionella*
  - Limited purulence and scanty secretions in patients with LD
  - Up to 80% of culture-positive specimens would have been rejected by microscopic scoring criteria

Author	Method	Criteria for Acceptability
Murray & Washington	Average no. of EPI/LPF	<10 EPI/LPF
Van Scoy	Average no. of WBC/LPF	>25 WBC/LPF
Barry	Assign + and - values, 3+ if > 150 WBC/LPF; 2+ if 76-150 WBC/LPF; 1+ if 1-75 WBC/LPF; -3 if >25 EPI/LPF; -2 if 16-25 EPI/LPF; -1 if 5-15 EPI/LPF	Any positive score (sum of + and - values)
Gal-oz	Informative: <10 SEC/LPF & >25 PMNs/LPF Semi-informative: <10 SEC/LPF or >25 PMNs/LPF Uninformative: >10 SEC/LPF & <25 PMNs/LPF	(Semi) informative considered to be





# *Legionella* Culture

- Isolation of all known *Legionella* species requires medium supplementation with L-cysteine
- BCYE: buffered-charcoal yeast extract
  - Activated charcoal to inactivate toxic lipids and peroxides
  - Organic buffer to reduce sodium content and provide required pH
- Sensitive in severe untreated disease (80-90%)
- Insensitive (~20%) in patients with mild disease



## Clicker question 2

Does your lab perform *Legionella* culture?

- A) Yes
- B) No
- C) I'll have to get back to you...

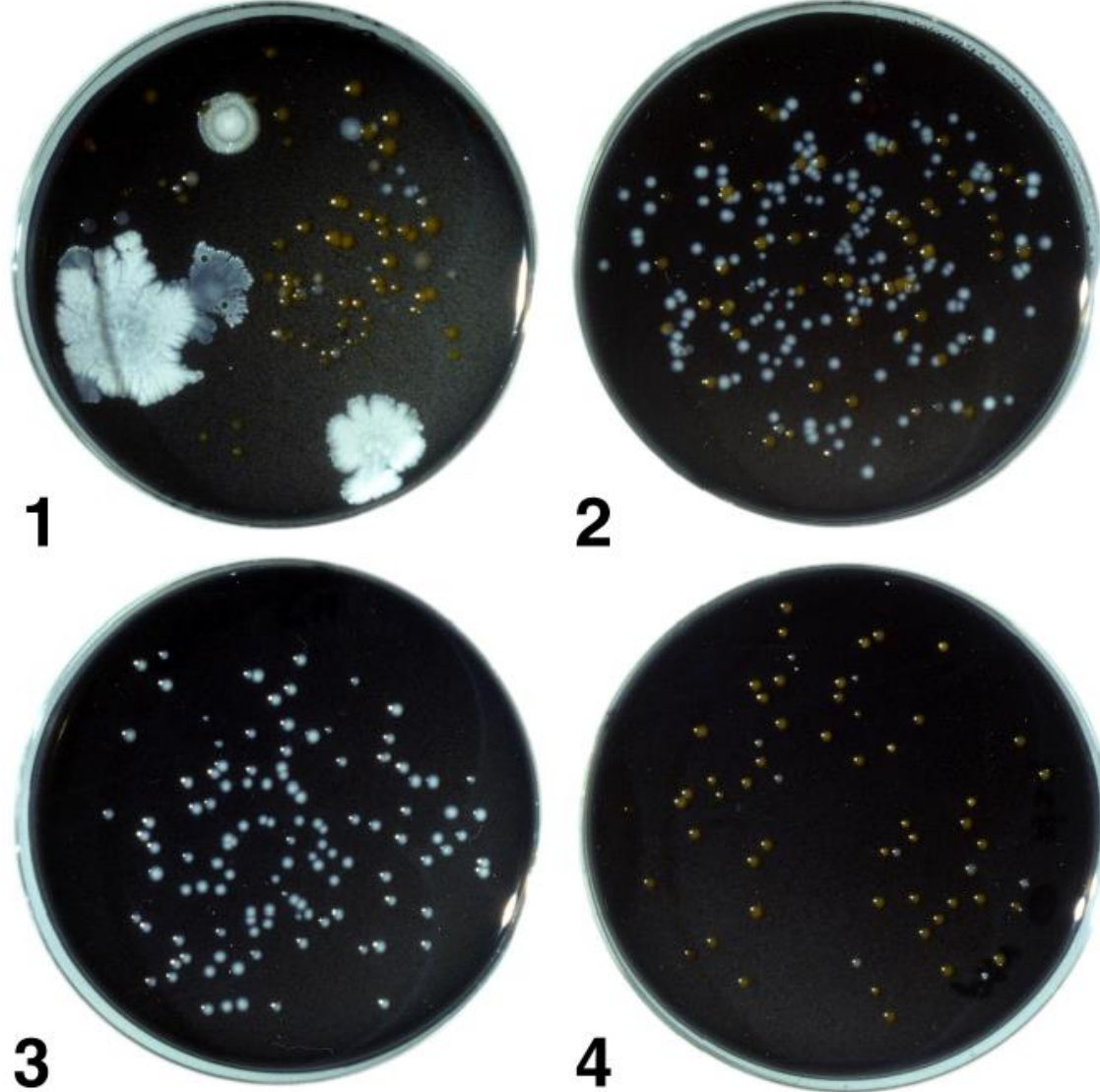


Figure 2. A water sample containing legionellae after culture on the four types of media. Plate 1, BCYE agar with numerous non-*Legionella* bacteria and a few *Legionella* colonies; Plate 2, PCV agar with numerous *Legionella* colonies and other bacteria; Plate 3, GPCV agar with *Legionella*, few, if any non-*Legionella* bacteria are present; Plate 4, PCV-without cysteine agar with some non-*Legionella* bacteria; no legionellae are present.



Figure 3. *Legionella* colonies as seen through a dissection microscope on primary isolation (4 days incubation). Note the white “cut-glass” appearance of the center of the colony and the purple iridescence which borders it. The iridescence can be one of several colors; the significance of the color is unknown.





## Clicker question 3

Which other microorganisms grow on BCYE media?

- A) *Nocardia* spp.
- B) *Blastomyces* spp.
- C) *Francisella tularensis*
- D) All of the above



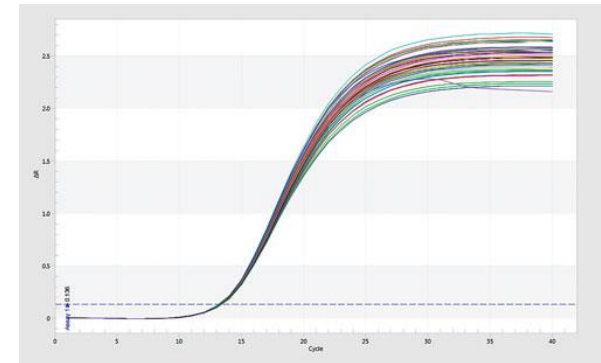
## Clicker question 3 – follow-up

- *F. tularensis*: the only other Gram-negative that exhibits L-cysteine growth dependence
- Some serotyping reagents for *Legionella* can cross-react with *F. tularensis*
  - One case of mis-identification of *F. tularensis* as LP has been published
- Colony morphologies of the two differ



# *L. pneumophila* real-time PCR

- Sensitive, specific, faster TAT than culture
- Developed by CDC
- Currently being validated at WSLH
  - Colonies
  - Specimens
- Three targets:
  - *Legionella* species
  - *Legionella pneumophila*
  - *Legionella pneumophila* serogroup 1





# *L. pneumophila* Treatment

- Prompt treatment cures 95-99% of otherwise healthy persons
- Untreated disease causes death in:
  - 15% of previously healthy patients
  - Up to 75% of severely immunocompromised
- Treatment needs to have good intracellular activity:
  - For mild LD:
    - erythromycin, clarithromycin, azithromycin, tetracycline, doxycycline, levofloxacin, ciprofloxacin, moxifloxacin
  - Azithromycin and levofloxacin: drugs of choice for severe disease and immunocompromised patients
  - Beta-lactams and aminoglycosides: sub-acceptable intracellular activity

# *Legionella* Ecology



Found naturally in fresh water but in insufficient quantities to cause disease

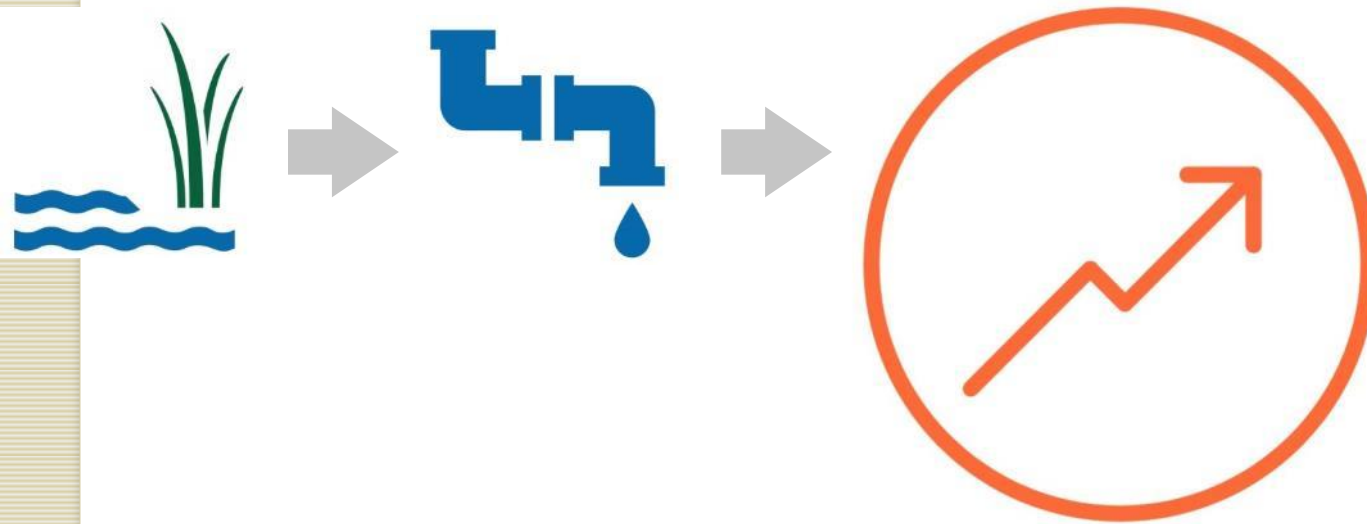
Grow in free-living protozoa in water

- Provide nutrients
- Protect from harsh environmental conditions

Can become a public health problem in human-made water systems



# Conditions for Transmission

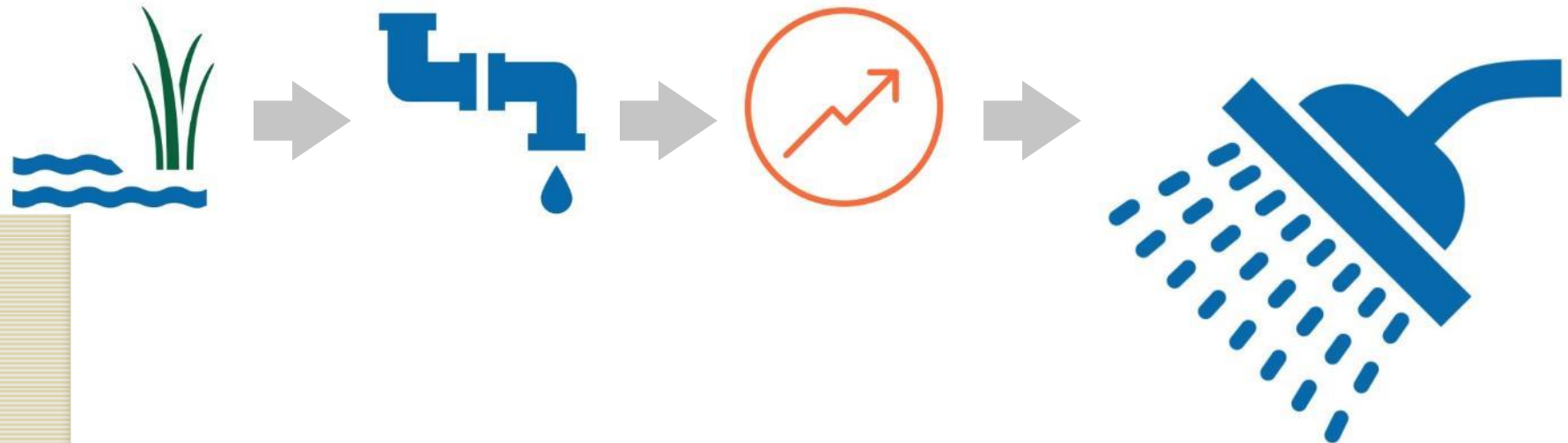


## Amplification

- Warm water (temperatures 77-108°F)
- Stagnation (dead legs in pipes)
- Sediment, scale, organic matter
- Absence of residual disinfectants in water supply
- Biofilm



# Conditions for Transmission



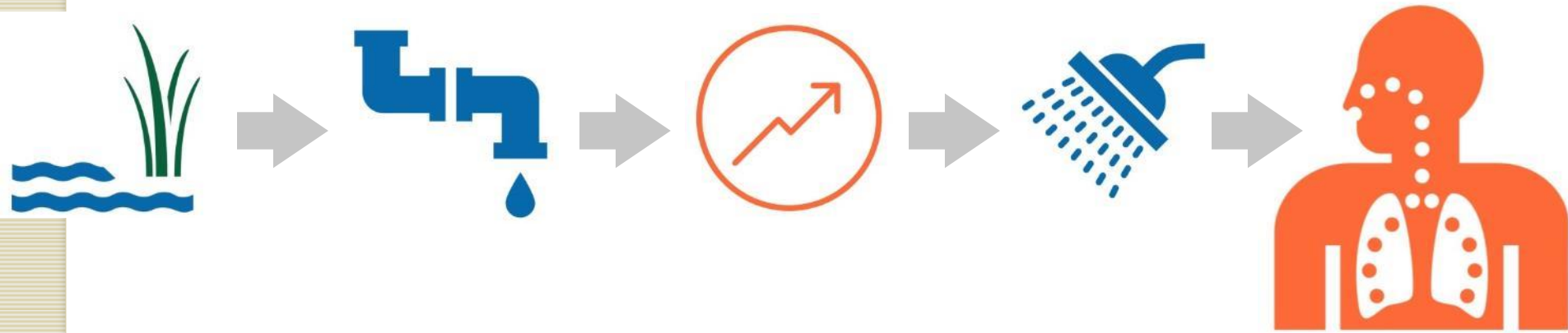
## Aerosolization

Devices that can aerosolize water droplets include:

- Showers and faucets
- Jetted hot tubs
- Decorative fountains
- Evaporative cooling towers (used in large buildings)



# Conditions for Transmission



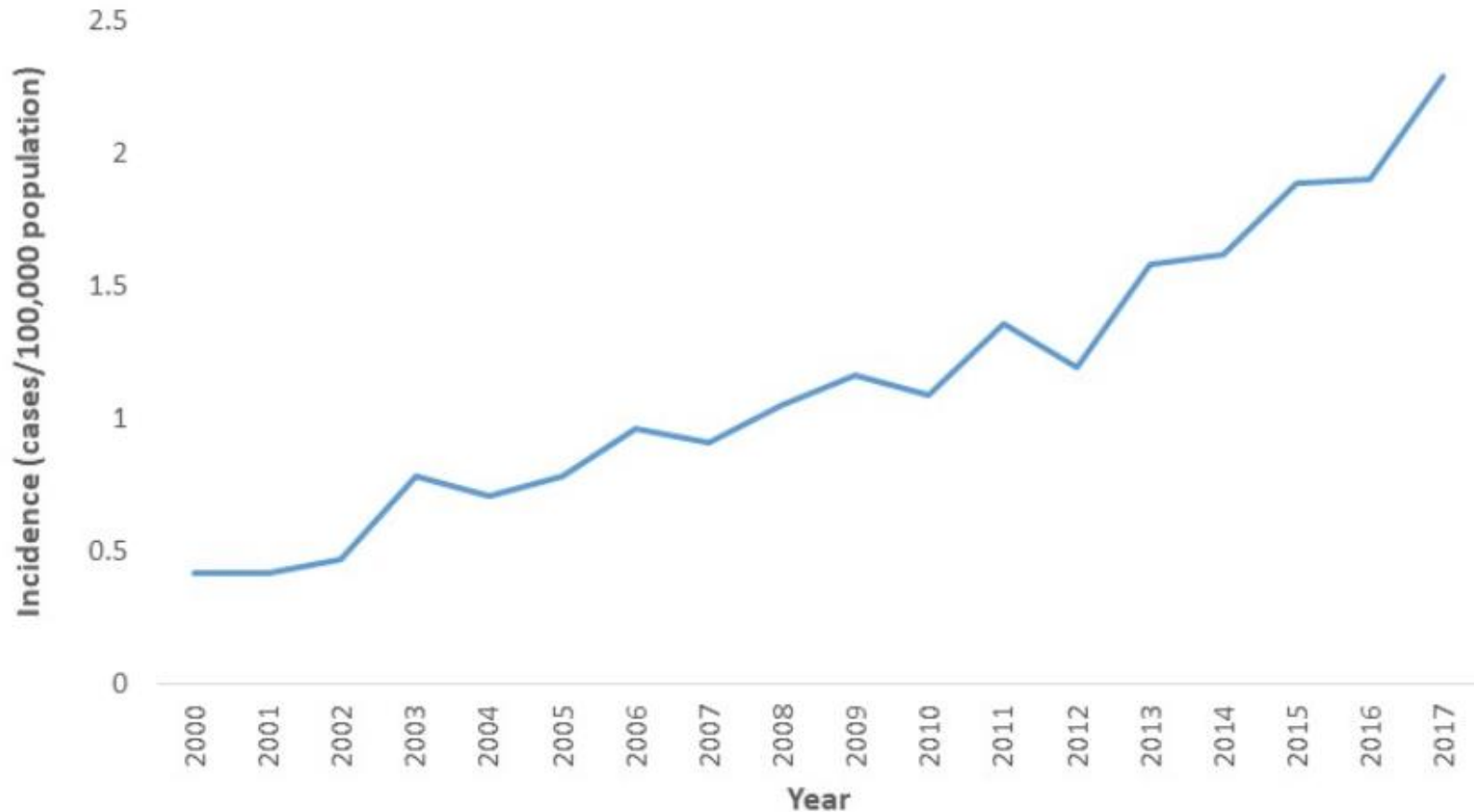
## Transmission

- Inhalation of aerosolized droplets, mists containing *Legionella*
- Aspiration (less common)
- **Not transmitted** from person to person





# National Incidence Trend



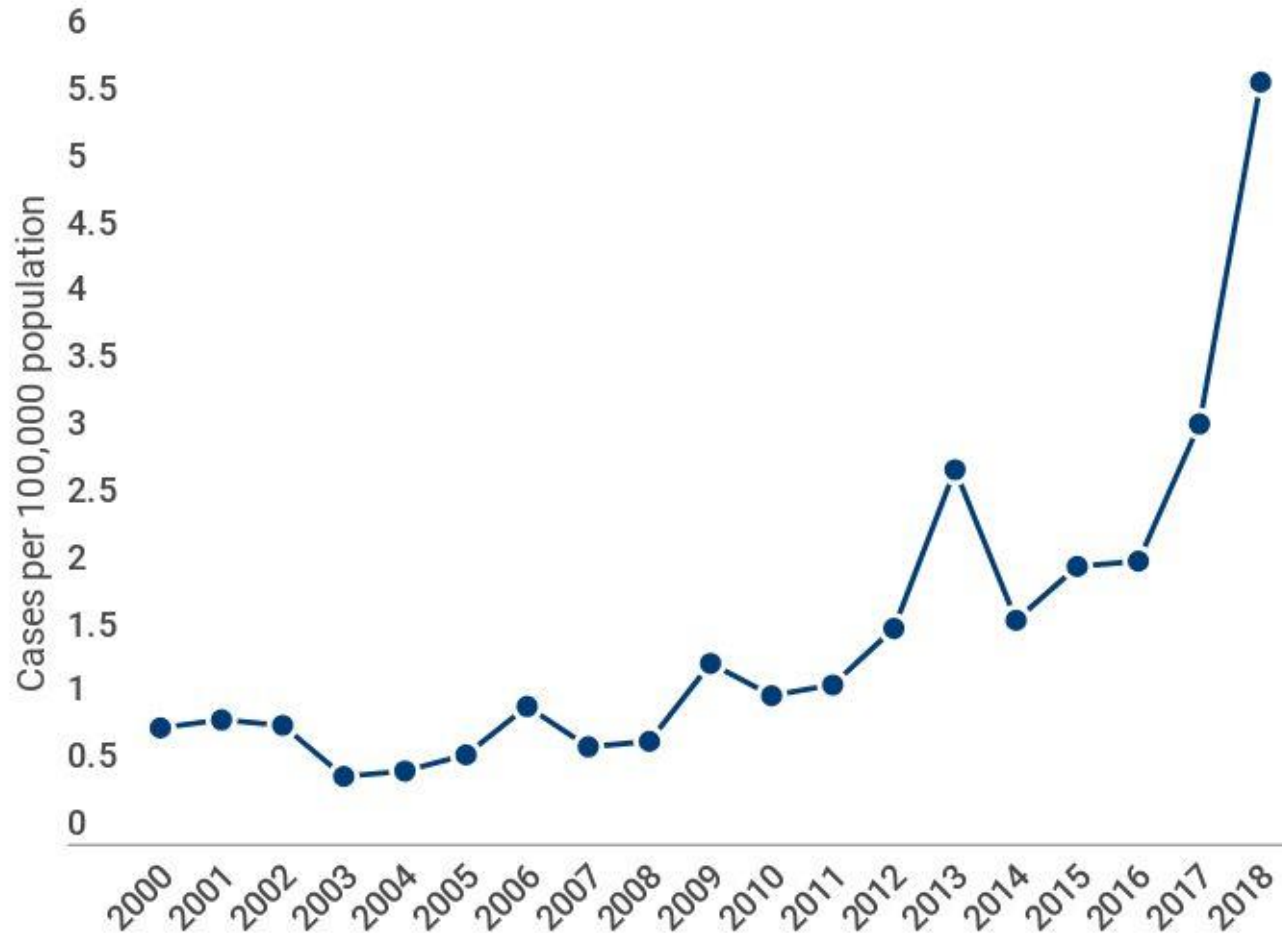
**Rate of reported cases increased 5.5 times (2000–2017)**

Source: National Notifiable Diseases Surveillance System



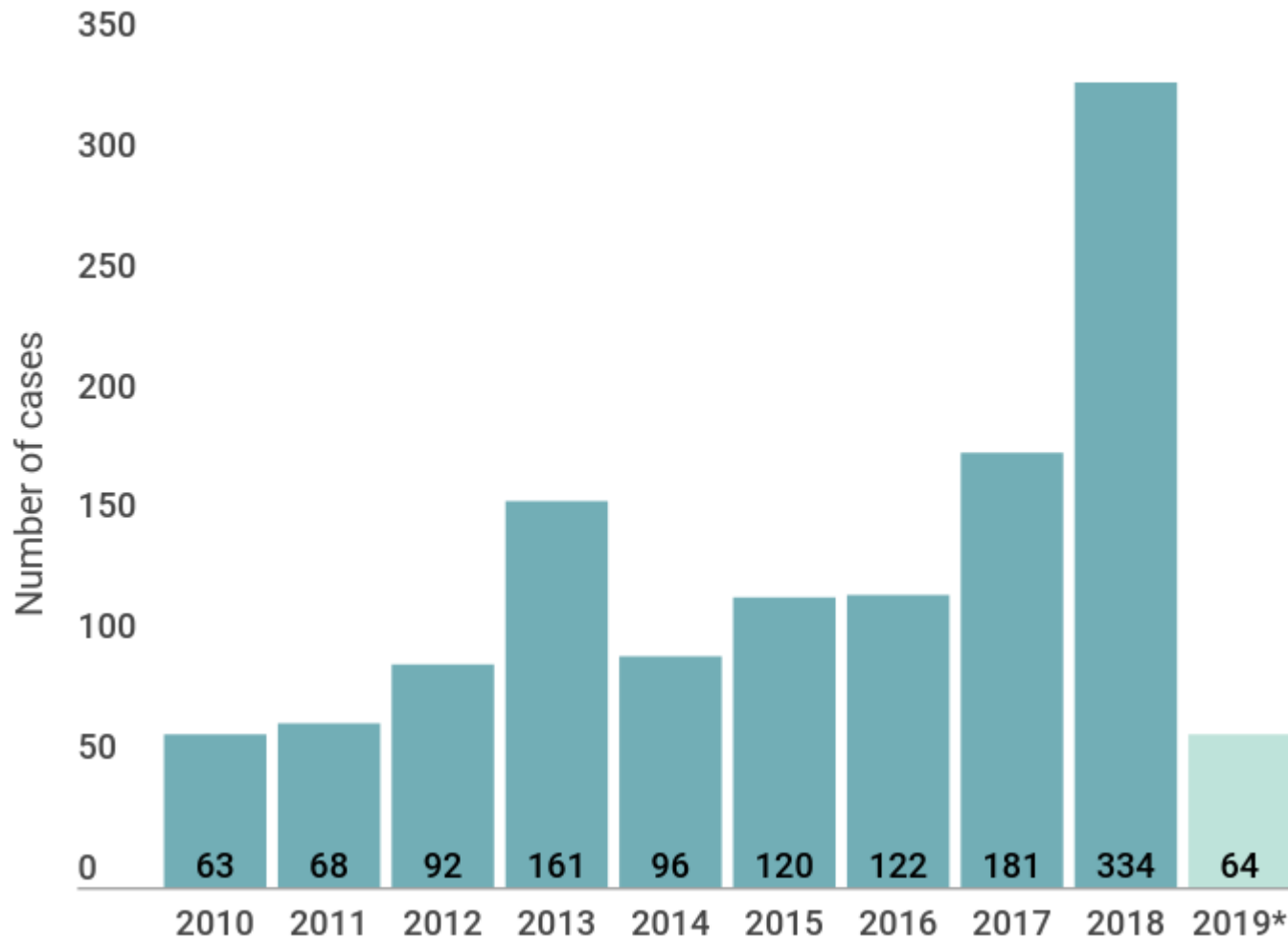
# Wisconsin Incidence Trend

Laboratory-confirmed cases, Wisconsin Electronic Disease Surveillance System



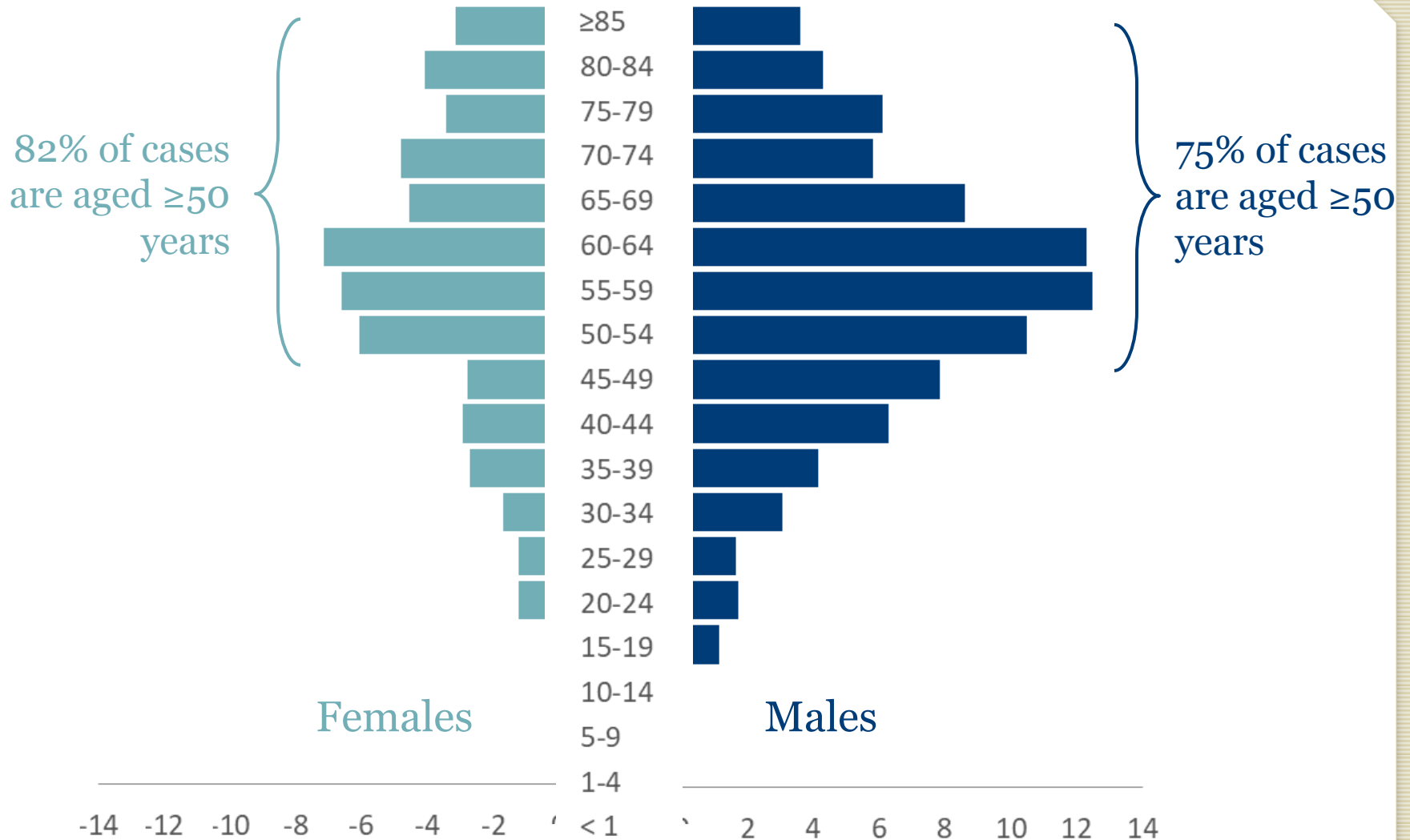


# Lab-confirmed Wisconsin Cases



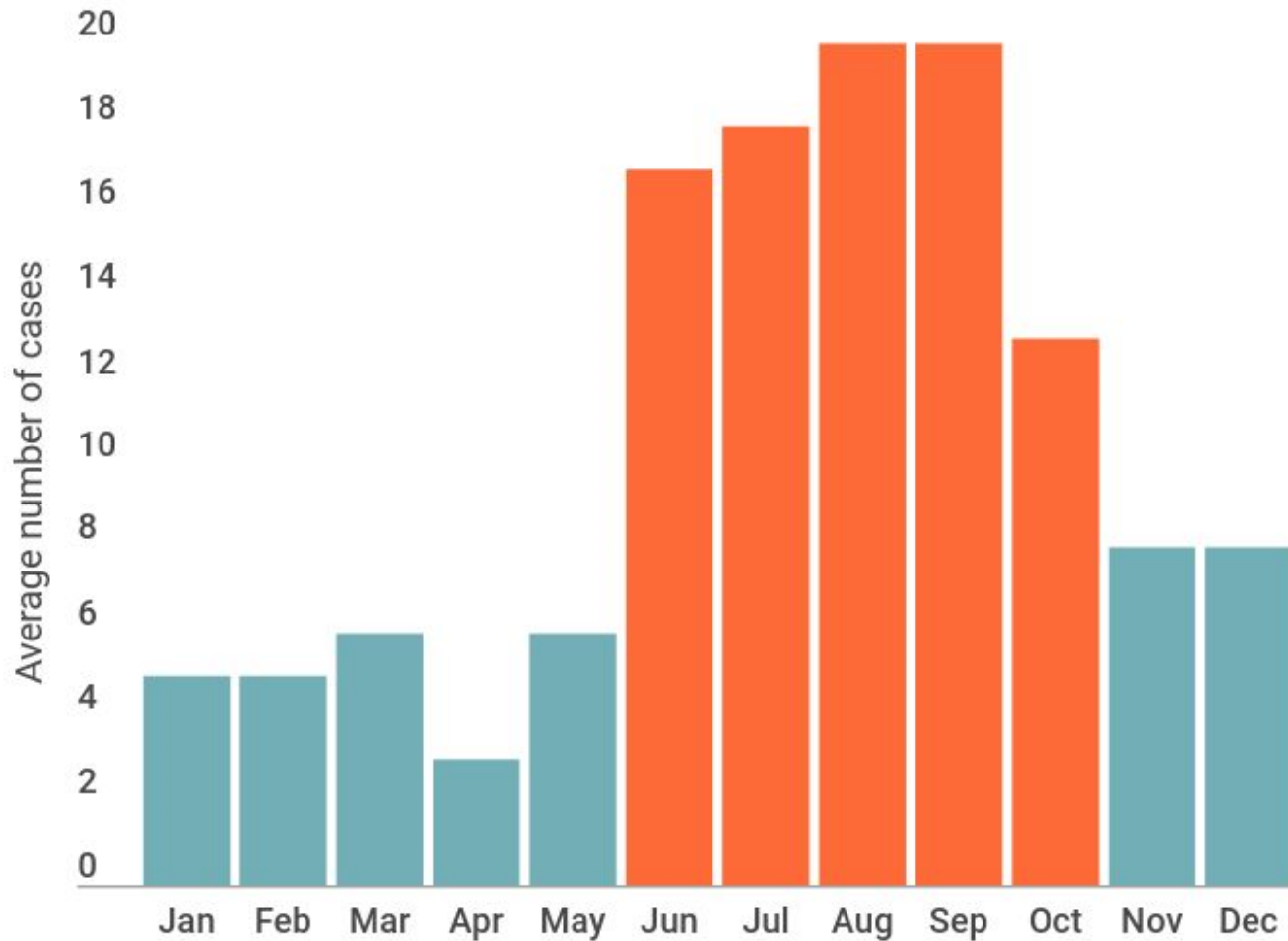


# Laboratory-confirmed cases by age group and gender, 2009-2018 average





## Laboratory-confirmed cases by month of illness onset, 2009-2018 average





**STATE OF WISCONSIN**  
Department of Health Services  
Division of Public Health



1 West Wilson Street  
PO Box 2659  
Madison WI 53701-2659

Telephone: 608-267-9003  
Fax: 608-261-4976  
TTY: 888-701-1253

Date: July 10, 2019

DPH Memo BCD-2019-10  
Replaces DPH Memo BCD-2018-09

To: Wisconsin Clinicians, Infection Preventionists, Laboratorians, Local Health Departments, and Tribal Health Agencies

From: Jon Meiman, MD  
Acting Chief Medical Officer and State Epidemiologist for Communicable Diseases

**Increased reports of laboratory-confirmed cases of legionellosis (Legionnaires' disease)**



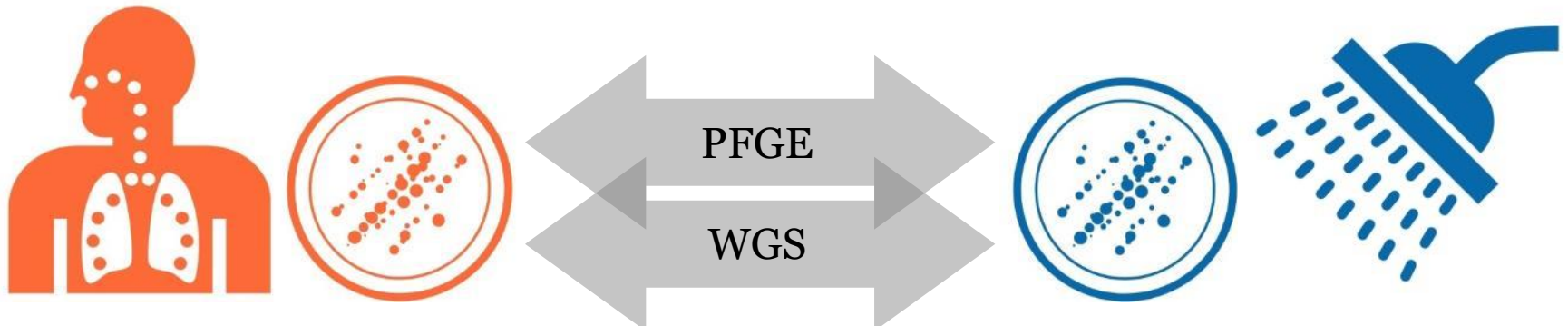
# DPH Memo

- Increase in *Legionella* in recent years
- Request assistance in diagnosing and reporting cases of Legionnaires' Disease
- For laboratorians:
  - Urinary antigen assay and culture of lower respiratory secretions on BCYE are the preferred diagnostic tests for Legionnaires' disease
    - Identify non-LP serogroup 1
    - Provide an isolate for subtyping (PFGE and/or WGS)



# DPH Memo, continued

- If your laboratory does not perform *Legionella* culture, send specimens to WSLH
  - DPH will approve fee-exempt testing for *Legionella* for patients meeting criteria
- For patients with a positive urine antigen test, send residual sputum or other lower respiratory specimens to the WSLH for *Legionella* culture
  - Provide an isolate for PFGE and/or WGS
- Forward all clinical *Legionella* isolates to the WSLH for PFGE and/or WGS
  - Link environmental isolates and clinical isolates
  - Mediate exposures, prevent future infections







# Example of *Legionella* Public Health Investigation and Response in Wisconsin



# Assisted Living Facility A

Case-patient A reported to public health on 7/17

62-year-old male

Onset of illness: 7/14

- Fever
- Chills
- Night sweats
- No appetite

**July, 2017**

Su	M	Tu	W	Th	F	Sa
25	26	27	28	29	30	1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31	1	2	3	4	5

Hospitalized 7/16 and diagnosed with pneumonia

*Legionella* urinary antigen positive on 7/16



## Case-patient A exposures:

Admitted to facility A on 6/30

Other exposures during incubation period:

- Swimming in a pool
- Showering at a fitness center
- Grocery shopping
- Attending church
- Visiting a salon

**July, 2017**

Su	M	Tu	W	Th	F	Sa
25	26	27	28	29	30	1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31	1	2	3	4	5



# Assisted Living Facility A

Case-patient B reported to public health on 8/28

89-year-old female

Onset of illness: 8/25

- Fever
- Cough
- Weakness and fatigue
- Decreased appetite

**August, 2017**

Su	M	Tu	W	Th	F	Sa
30	31	1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31	1	2
3	4	5	6	7	8	9

Hospitalized 8/27 and diagnosed with pneumonia

*Legionella* urinary antigen positive on 8/27

Sputum collected 8/27 positive by culture on 9/7



## Case-patient B exposures:

Admitted to facility A on 8/9

Other exposures during incubation period:

- Possibly shopping

(Unable to interview patient right away to determine additional exposures)

August, 2017						
Su	M	Tu	W	Th	F	Sa
30	31	1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31	1	2
3	4	5	6	7	8	9



# Assisted Living Facility A

## Environmental Investigation

Environmental  
assessment



- Municipal water not disinfected
- Hot water heater temperature settings
- No cooling tower/evaporative condenser
- No pool or whirlpool spa (single-use tub only)

8/25 8/27 8/29 8/31 9/2 9/4 9/6 9/8 9/10 9/12 9/14 9/16 9/18 9/20 9/22 9/24 9/26 9/28 9/30 10/2 10/4 10/6



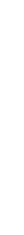
# Assisted Living Facility A

## Environmental Investigation

Environmental  
assessment



Environmental  
sampling



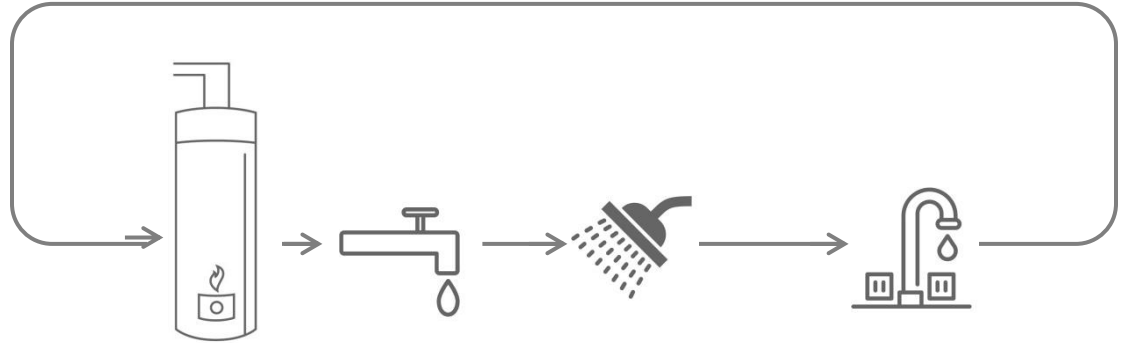
8/25 8/27 8/29 8/31 9/2 9/4 9/6 9/8 9/10 9/12 9/14 9/16 9/18 9/20 9/22 9/24 9/26 9/28 9/30 10/2 10/4 10/6



# Environmental Sampling

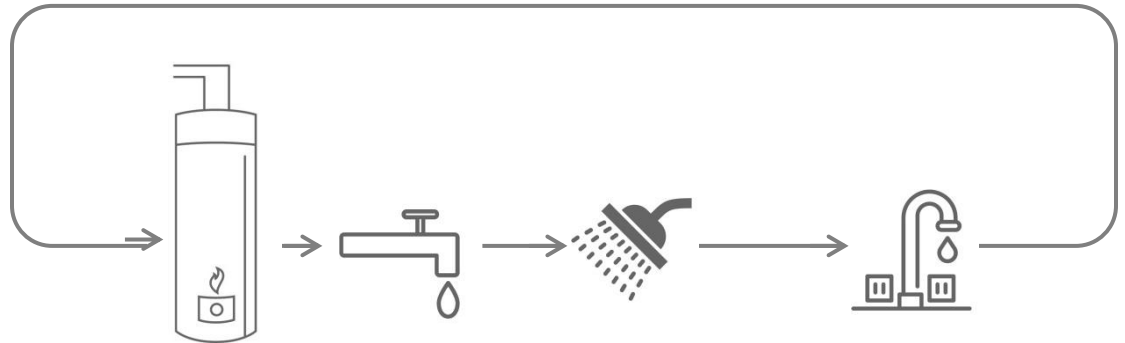
## Third floor (patient A room)

- Water heater tank
- Bathroom faucet
- Shower
- Kitchen faucet



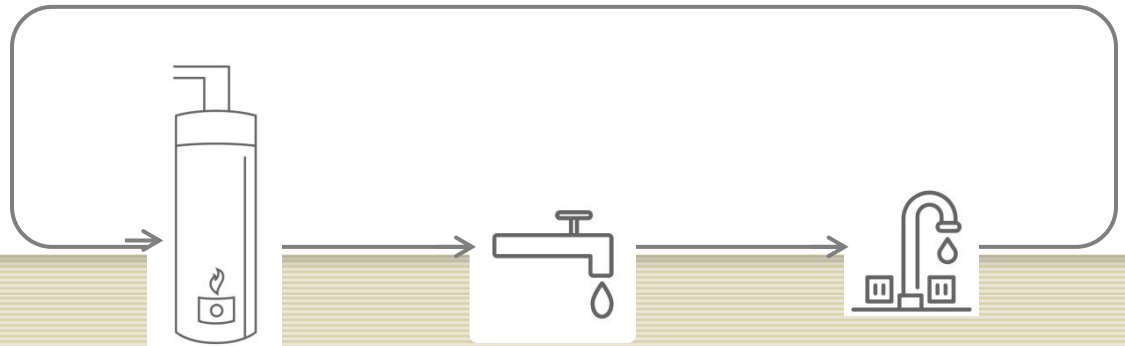
## Second floor (patient B room)

- Water heater tank
- Bathroom faucet
- Shower
- Kitchen faucet



## Main floor

- Water heater tank
- Bathroom faucet
- Kitchen faucet

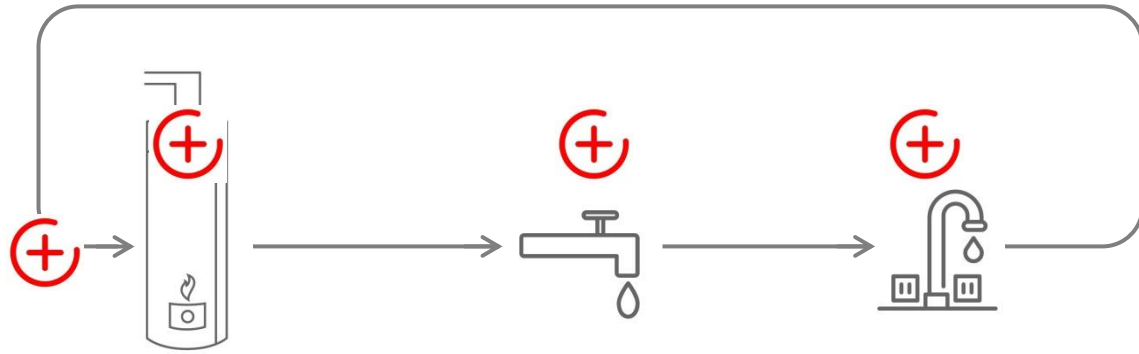
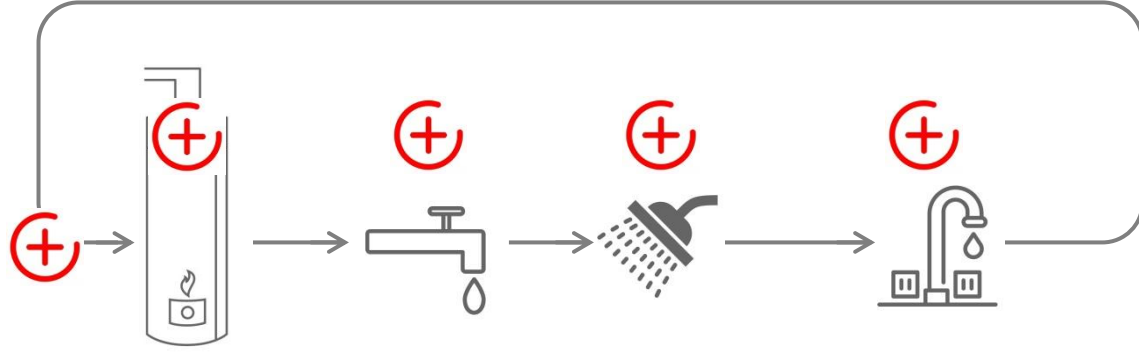
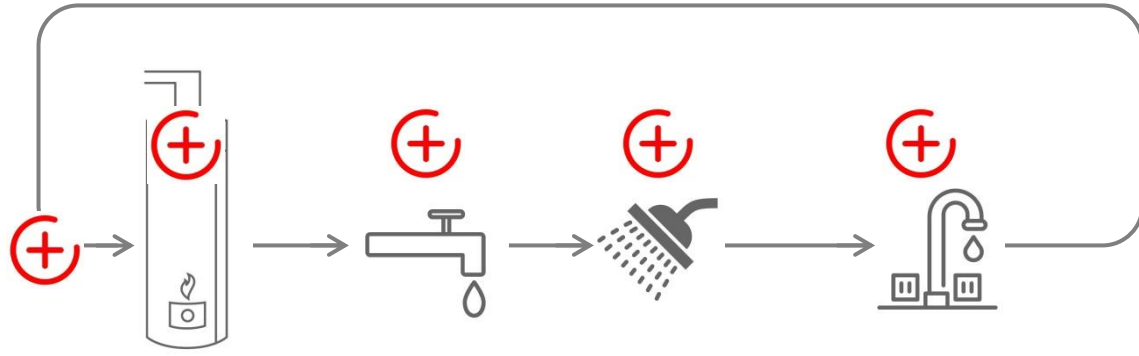






# Environmental Testing Results

Sample ID	Date Collected	Specimen Type	Sample description	Temp (°F)	Free Cl <sub>2</sub> (ppm)	pH	Result	Count	Concentration, bulk water (CFU/ml)	Concentration, swab (CFU/sample)
001	9/12/2017	Swab	Room B (patient B), shower		0.0		<i>Legionella pneumophila</i>	1		13
002	9/12/2017	Bulk water	Room B, shower		0.0		<i>Legionella pneumophila</i>	3	0.15	
003	9/12/2017	Swab	Room B, bathroom faucet		0.0		No <i>Legionella</i> isolated	0		<13
004	9/12/2017	Bulk water	Room B, bathroom faucet		0.0		<i>Legionella pneumophila</i>	6	0.3	
005	9/12/2017	Swab	Room B, kitchen faucet		0.0		<i>Legionella pneumophila</i>	4		50
006	9/12/2017	Bulk water	Room B, kitchen faucet	112.0	0.0	7.2	<i>Legionella pneumophila</i>	4	0.2	
007	9/12/2017	Bulk water	Room B, water heater tank		0.0		<i>Legionella pneumophila</i>	2,080	100	
008	9/12/2017	Swab	Room A (patient A), shower		0.0		<i>Legionella pneumophila</i>	45		560
009	9/12/2017	Bulk water	Room A, shower		0.0		<i>Legionella pneumophila</i>	98	4.9	
010	9/12/2017	Swab	Room A, bathroom faucet		0.0		<i>Legionella pneumophila</i>	55		690
011	9/12/2017	Bulk water	Room A, bathroom faucet		0.0		<i>Legionella pneumophila</i>	37	1.9	
012	9/12/2017	Swab	Room A, kitchen faucet		0.0		<i>Legionella pneumophila</i>	5		63
013	9/12/2017	Bulk water	Room A, kitchen faucet	113.0	0.0	7.4	<i>Legionella pneumophila</i>	56	2.8	
014	9/12/2017	Bulk water	Room A, water heater tank		0.0		<i>Legionella pneumophila</i>	563	37	
015	9/12/2017	Bulk water	Main floor, shared water heater		0.0		<i>Legionella pneumophila</i>	130	6.5	
016	9/12/2017	Swab	Main floor, shared kitchen faucet		0.0		<i>Legionella pneumophila</i>	3		38
017	9/12/2017	Bulk water	Main floor, shared kitchen faucet		0.0		<i>Legionella pneumophila</i>	53	2.7	
018	9/12/2017	Swab	Main floor, shared bathroom faucet		0.0		<i>Legionella pneumophila</i>	3,200		53,000
019	9/12/2017	Bulk water	Main floor, shared bathroom faucet		0.0		<i>Legionella pneumophila</i>	67	3.4	





# Interpretation of Environmental Testing Results

## Locations of positive samples

- Systemwide versus localized to distal points
- In conjunction with environmental assessment

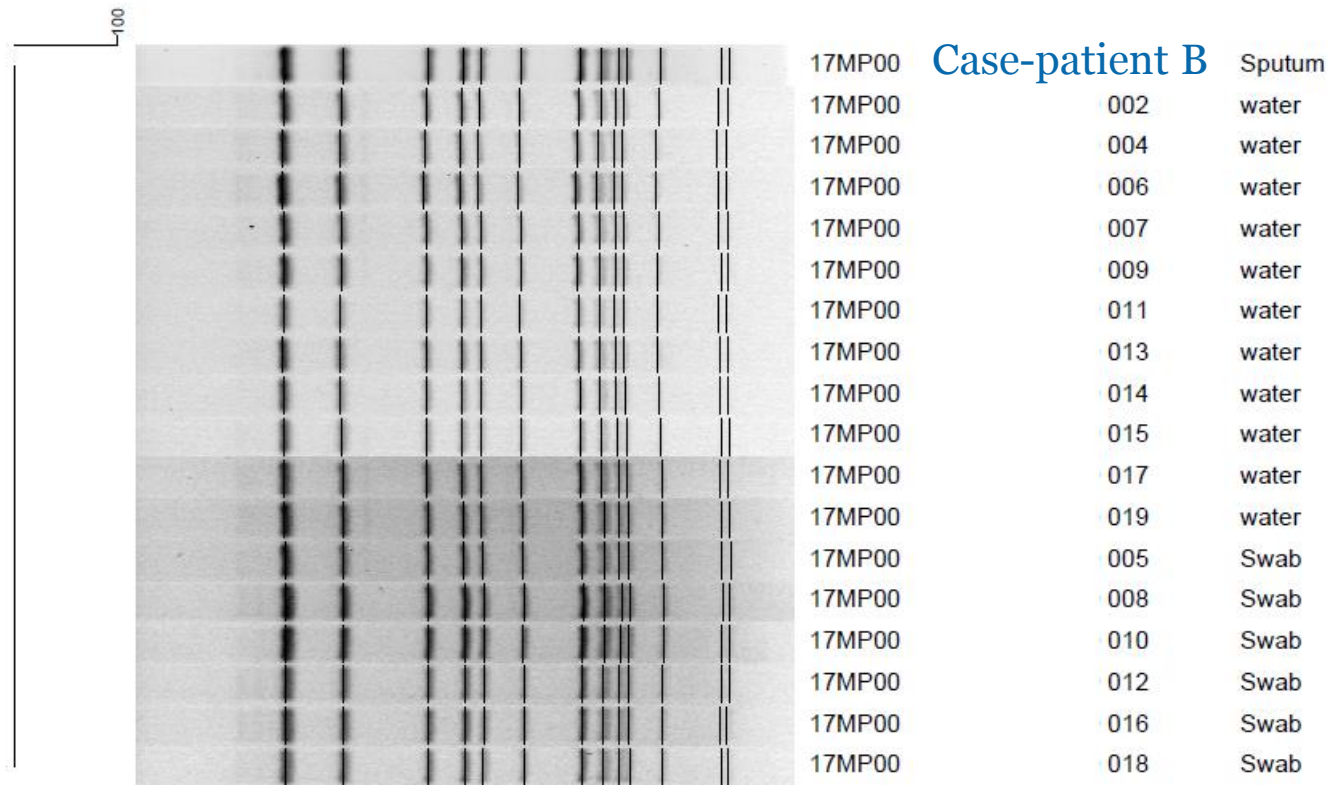
## Presence versus absence of *Legionella* within water system

- *Legionella pneumophila*
- Other *Legionella* species
- Molecular subtyping and comparison with clinical samples



# Linking Clinical and Environmental Isolates

## PFGE at WSLH





# Whole-genome Sequencing

1. Isolate DNA
2. Library preparation
  - 200-300bp fragments
3. Sequence
  - Illumina MiSeq
  - Millions of 'reads'
4. Analyze
  - SNP
  - wgMLST
  - many others

```
ggaatagaacagatTTTGGCAAAAATATCCACAGCGGCTGTGGagataattgagacagttcag
GAACAGATTTTGGCAAAAATATCCACAGCGGCTGTGG
ACAGATTTTGGCAAAAATATCCACAGCGGCTGTGG
ACAGATTTTGGCAAAAATATCCACAGCGGCTGTGG
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AATATCCACAGCGGCTGTGGGGATAATT
ATATCCACAGCGGCTGTGGGGATAATT
ATCCACAGCGGCTGTGGGGATAATT
TCCACAGCGGCTGTGGGGATAATT
ACAGCGGCTGTGGGGATAATTGAGA
```

Comparison to analyzing a book:

- PFGE tells you the length of each chapter
- WGS tells you every letter in the book



# Public Health Applications of Whole-genome Sequencing

## Advanced molecular fingerprinting to detect outbreaks

- PulseNet
  - *Salmonella*, STEC, *Listeria*
- AR organisms
  - CP-CRE, CP-PA, CRAB, *Stenotrophomonas*, VRE, etc.
- Influenza
- *Cyclospora*
- Hepatitis C virus
- *M. tuberculosis*
- *Legionella*



# Linking Clinical and Environmental Isolates

- WGS plus wgMLST analysis at CDC
- This year: sequencing at WSLH, analysis at CDC/WSLH

Year	Sample_ID	Serogroup	Source	ST	State	Comments
2018		3	Environmental	93*	WI	
2018		3	Environmental	93*	WI	
2018		1	Environmental	40*	WI	Rm water heater tank
2018		1	Environmental	40*	WI	Rm water heater tank
2018		1	Environmental	40*	WI	Rm shower

Clinical isolate linked to these environmental isolates



# Response to mitigate risk and prevent further infections

- Notified residents, staff, and families
- Water restriction measures (for example, showering)
- Installed point-of-use filters on faucets
- Continued enhanced surveillance for legionellosis
- Restricted new admissions
- Remediated water system with assistance from specialized consultants
- Development of water management plan

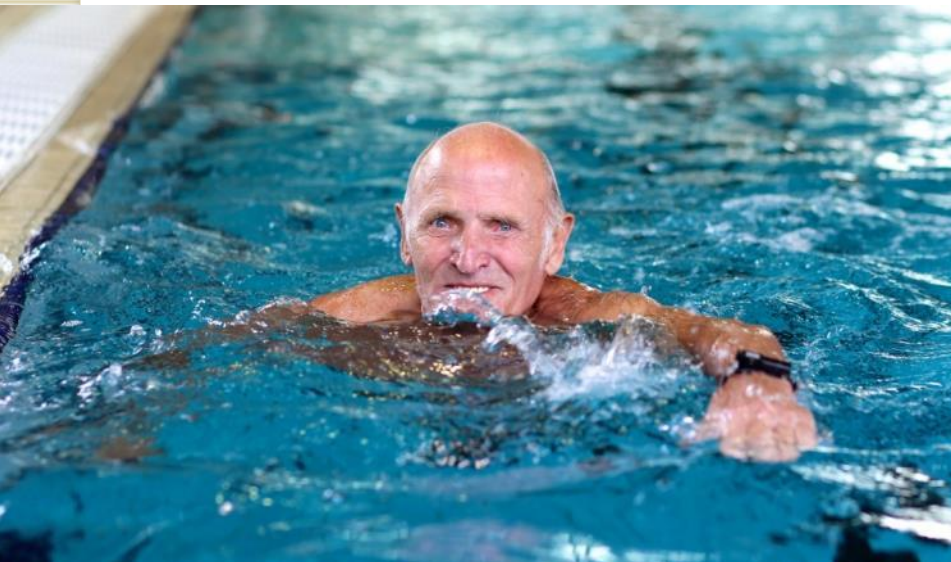




Questions?



# Possible Reasons for Rising Trends



Increased susceptibility



*Legionella* in environment



Improved diagnostics



Improved surveillance



# Increased risk for Legionnaires' disease

- Are aged 50 years and older.
- Are current or former smokers.
- Have chronic lung disease.
- Have a weakened immune system.



## **Legionnaires' disease      Pontiac fever**

Signs and symptoms

Pneumonia

No pneumonia

Cough, fever, muscle aches, shortness of breath, chest pain, headache, confusion, diarrhea

Mild, self-limiting illness with fever and muscle aches

Incubation period

2–10 days (up to 2 weeks)

24–72 hours

Attack rate

< 5%

> 90%

Treatment

Antibiotics

Supportive care

Hospitalization

Common

Uncommon

Case-fatality rate

**10%** (>25% for healthcare-associated infections)

**Extremely low**