Fastidious Gram-negative bacteria

Bacterial vaginosis, HACEK infections, Legionella

Prof. Cary Engleberg, M.D.
Division of Infectious Diseases,
Department of Internal Medicine
Dept. of Microbiology & Immunology

Unless otherwise noted, this material is made available under the terms of the **Creative Commons Attribution 3.0 License**: http://creativecommons.org/licenses/by/3.0/

Disclaimers

- I have reviewed this material in accordance with U.S. Copyright Law and have tried to maximize your ability to use, share, and adapt it. The citation key on the following slide provides information about how you may share and adapt this material.
- Copyright holders of content included in this material should contact open.michigan@umich.edu with any questions, corrections, or clarification regarding the use of content.
- For more information about how to cite these materials visit http://open.umich.edu/education/about/terms-of-use.
- Any medical information in this material is intended to inform and educate and is not a tool for self-diagnosis or a replacement for medical evaluation, advice, diagnosis or treatment by a healthcare professional. Please speak to your physician if you have questions about your medical condition.
- Viewer discretion is advised: Some medical content is graphic and may not be suitable for all viewers.

Citation Key

for more information see: http://open.umich.edu/wiki/CitationPolicy

Use + Share + Adapt

{ Content the copyright holder, author, or law permits you to use, share and adapt. }

Public Domain – Government: Works that are produced by the U.S. Government. (17 USC § 105)

Public Domain – Expired: Works that are no longer protected due to an expired copyright term.

Public Domain – Self Dedicated: Works that a copyright holder has dedicated to the public domain.

Creative Commons – Zero Waiver

Creative Commons – Attribution License

Creative Commons – Attribution Share Alike License

Creative Commons – Attribution Noncommercial License

Creative Commons – Attribution Noncommercial Share Alike License

GNU – Free Documentation License

Make Your Own Assessment

{ Content Open.Michigan believes can be used, shared, and adapted because it is ineligible for copyright. }

Public Domain – Ineligible: Works that are ineligible for copyright protection in the U.S. (17 USC § 102(b)) *laws in your jurisdiction may differ

{ Content Open.Michigan has used under a Fair Use determination. }

Fair Use: Use of works that is determined to be Fair consistent with the U.S. Copyright Act. (17 USC § 107) *laws in your jurisdiction may differ

Our determination **DOES NOT** mean that all uses of this 3rd-party content are Fair Uses and we **DO NOT** guarantee that your use of the content is Fair.

To use this content you should **do your own independent analysis** to determine whether or not your use will be Fair.

Gram-negative normal flora

Gardenerella vaginalis — associated with bacterial vaginosis

Mobiluncus spp. — associated with bacterial vaginosis

HACEK (including *Actinobacillus*) — rare causes of endocarditis; (juvenile periodontitis)

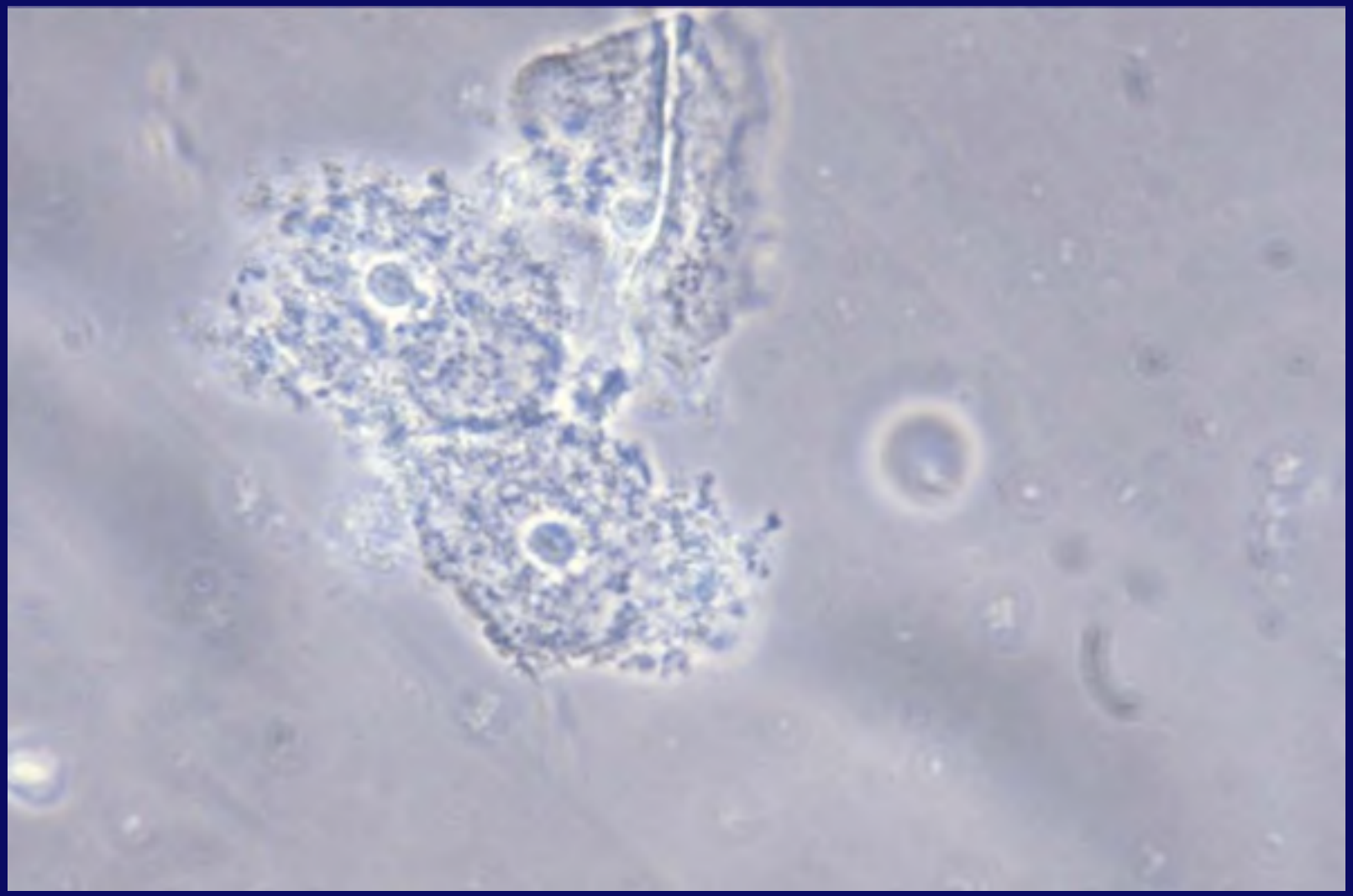
Gram-negative, intracellular pathogens, acquired from the environment

Legionella spp. (*L. pneumophila*) — severe pneumonia (Legionnaires' disease)

Case: vaginitis

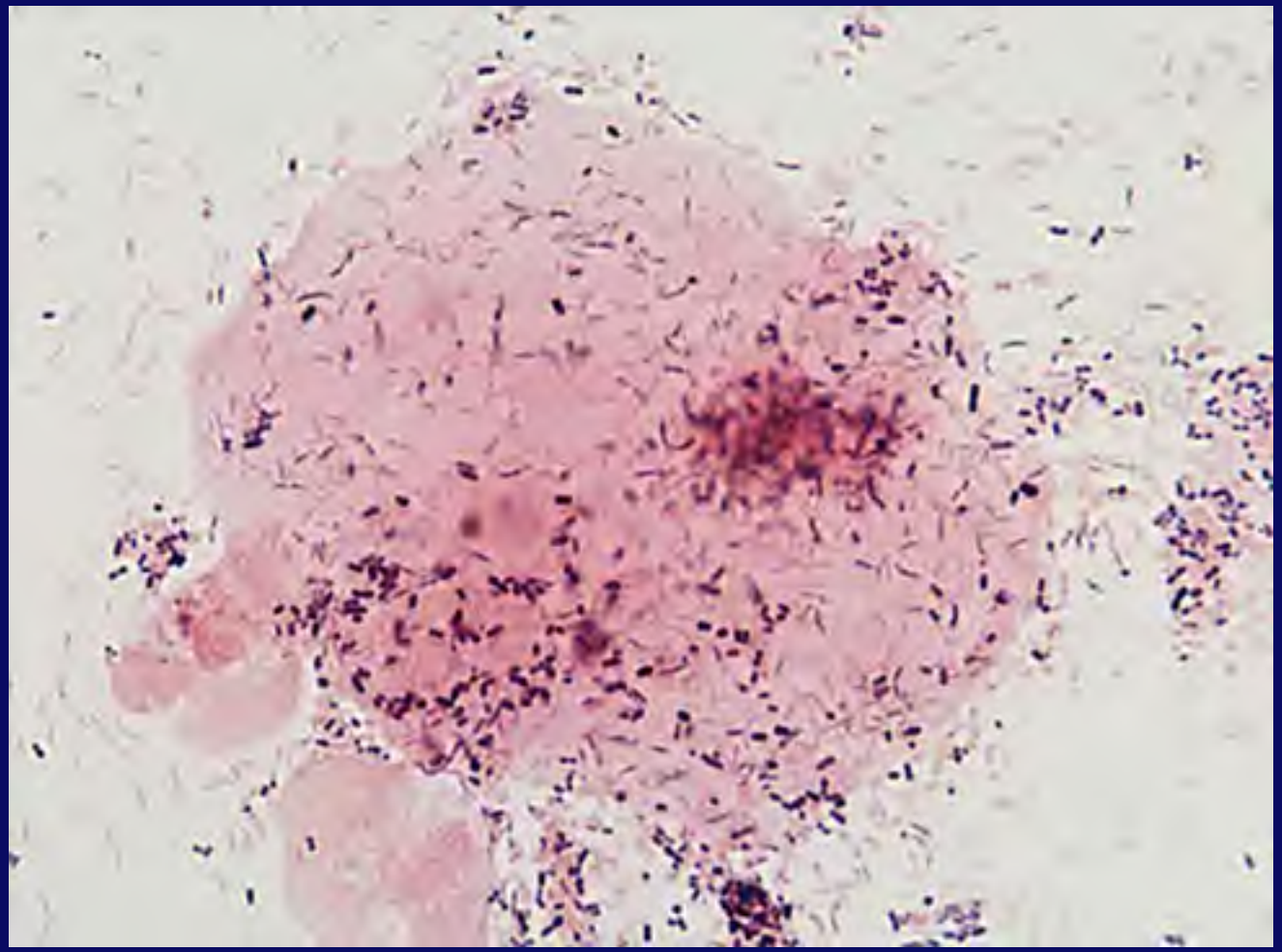
- A 32 year old woman has a vaginal discharge. She has had no pain, vaginal bleeding, or excessive weight gain. She is G2P2. LMP was two weeks ago and was normal and on time. She is monogamous with her husband.
- On examination, there is a malodorous, light gray discharge at the vaginal introitus.
- Application of a pH strip to vaginal wall =7.0.
- Addition of 10% KOH to a sample of the discharge on a slide produces an intense amine odor ("fishy"). A saline preparation of the discharge shows the following:

“Clue” cells



PD-GOV M Rein, CDC Public Health Image Library, #3720

Clue cell Gram stain



PD-INEL Source undetermined

Questions to consider

- Why is the discharge malodorous? What is the "fishy" odor?
- What is the significance of the vaginal pH?
- What is the significance of bacteria-coated cells ("clue" cells)?
- Should her husband be examined and treated?

Bacterial vaginosis (BV)

- Overgrowth of vaginal flora with *G. vaginalis*, *Mobiluncus*, *Prevotella*, *Peptostreptococcus*, and many other anaerobic species
- Displaces normal lactobacilli (responsible for vaginal acid production; pH<4.5)
- Anaerobic bacteria produce amines which release ammonia in 10% KOH
- Resolves with oral or topical metronidazole

Gardenerella vaginalis

- facultatively anaerobic, nonsporulating, nonencapsulated, nonmotile, pleomorphic, gram-variable rod
- found in 15% - 69% of women without BV and in 13.5% of girls.
- found in all cases of BV
- risk of bacteremia in pregnant women, post-abortion, and post-hysterectomy

Mobiluncus spp.

- anaerobic, slowly growing, motile, Gram-variable, curved bacilli
- found in 97% of women with BV but in a minority of healthy controls
- susceptible to most antibiotics, but *resistant* to metronidazole
  - ? role in BV

What is the role of these bacteria in BV?

- unclear. . .
- Koch's postulates not satisfied by any single agent
- Note: BV is not sexually-transmitted disease
- When is it necessary to treat?
  - HIV-infected
  - Pregnant
  - Before GYN surgery
  - At risk for other STDs

Questions to consider

- Why is the discharge malodorous? What is the "fishy" odor?
- What is the significance of the vaginal pH?
- What is the significance of bacteria-coated cells ("clue" cells)?
- Should her husband be examined and treated?

The HACEK group

H: *Haemophilus aphrophilus* and *H. paraphrophilus*

A: *Actinobacillus actinomycetemcomitans*

C: *Cardiobacterium hominis*

E: *Eikonella corrodens*

K: *Kingella kingii*

Characteristics of HACEK

- all are Gram-negative, pleomorphic rods
- all are normal flora of the human mouth
- rare cause of "culture-negative" endocarditis (particularly after dental work)
- fastidious; very slow to grow in culture (require 5-10% CO_2)
- most have beta-lactamase enzymes

Actinobacillus

- Found in 20% of adult/teenage mouths
- Cause of juvenile and adult periodontitis
  - with *Porphyromonas gingivalis*
  - In 90% localized, aggressive periodontal infections with loss of teeth and bone
- Can cause infections mimicking Actinomycosis (usually neck, face, lungs and chest wall) - rare

“Juvenile periodontitis”

PD-INEL Erste-zahnartzmeinung.de

Treatment of HACEK organisms

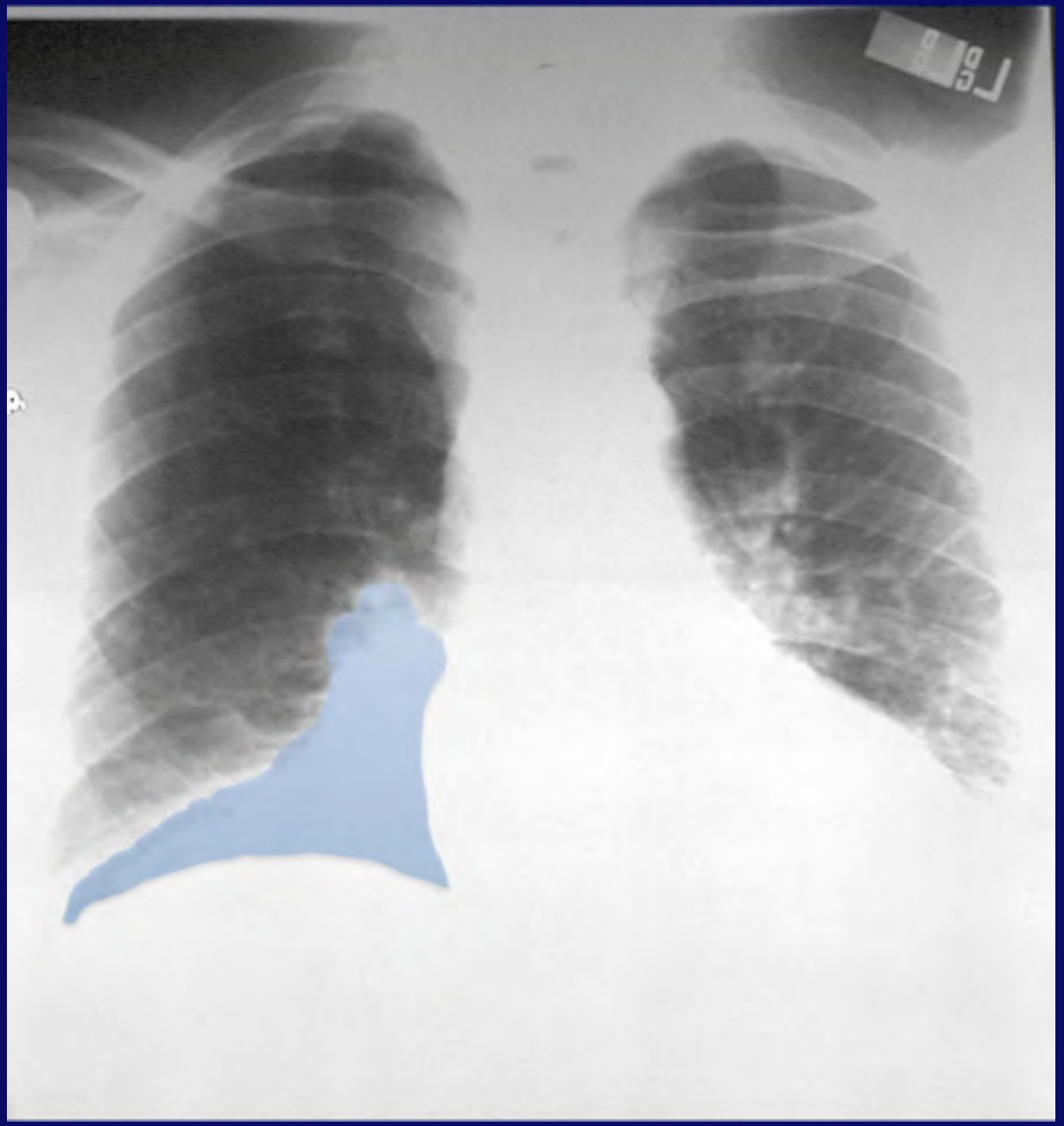
- Endocarditis: ceftriaxone, *not* ampicillin or penicillin
- Human bites: amoxicillin-clavulanate, fluoroquinolone
- Severe periodontitis: tetracycline

Legionella
(primarily *L. pneumophila*)

- Legionnaires' disease
- Pontiac Fever

Case: pneumonia after travel

- A 55-year-old male automobile dealer was hospitalized with high fever and cough.
- Seven days earlier, he developed symptoms of fever (38.5C), headache, and generalized muscle ache. The following day, he developed a hacking cough with minimal sputum production.
- He was evaluated 4 days before admission, and a diagnosis of community-acquired pneumonia was made after a chest x-ray which a left lower lobe infiltrate.



PD-INEL Source undetermined

Case (continued)

- He was treated with an oral cefuroxime. However, his fever increased, and he developed watery diarrhea.
- His past medical history was unremarkable, but he is a cigarette smoker. He returned from a 2-week vacation with his wife, three children, 75 year old mother in the Florida Keys (Hawthorne Suites) 2 days before onset. None of his family or co-workers were also ill.
- On admission, temp=39.8^{o}C, heart rate =90/minute. O_2 saturation=81% on room air
- WBC=13,700/mm^3.
- Sputum Gram stain: numerous PMNs, no bacteria.
- A chest x-ray showed extension of the right lower lobe infiltrate and an extensive new left lower and left upper lobe infiltrate.

PD-INEL

Source undetermined

Case (conclusion)

- Azithromycin was added to the patient's antibiotic regimen to treat Legionnaires' disease. This diagnosis was confirmed by a positive *Legionella* urine antigen test.
- The patient began to improve 48 hours later and recovered. Respiratory secretions obtained from his endotracheal tube grew *L. pneumophila* SG1 after 3 days.
- A call to the Florida Department of Public Health confirmed that 5 other recent patrons of the Hawthorne Suites had also developed severe pneumonia, and a hot tub at the hotel was positive for *L. pneumophila* SG1.

Questions to consider

- Was the hot tub the source of the illness? Why?
- Why were none of the patient's family members or co-workers affected?
- Why was the sputum Gram stain negative?
- Why was cefuroxime ineffective?
- Why was the diagnosis made by a urine antigen test instead of a culture?

The Belleview-Stratford Hotel, Philadelphia

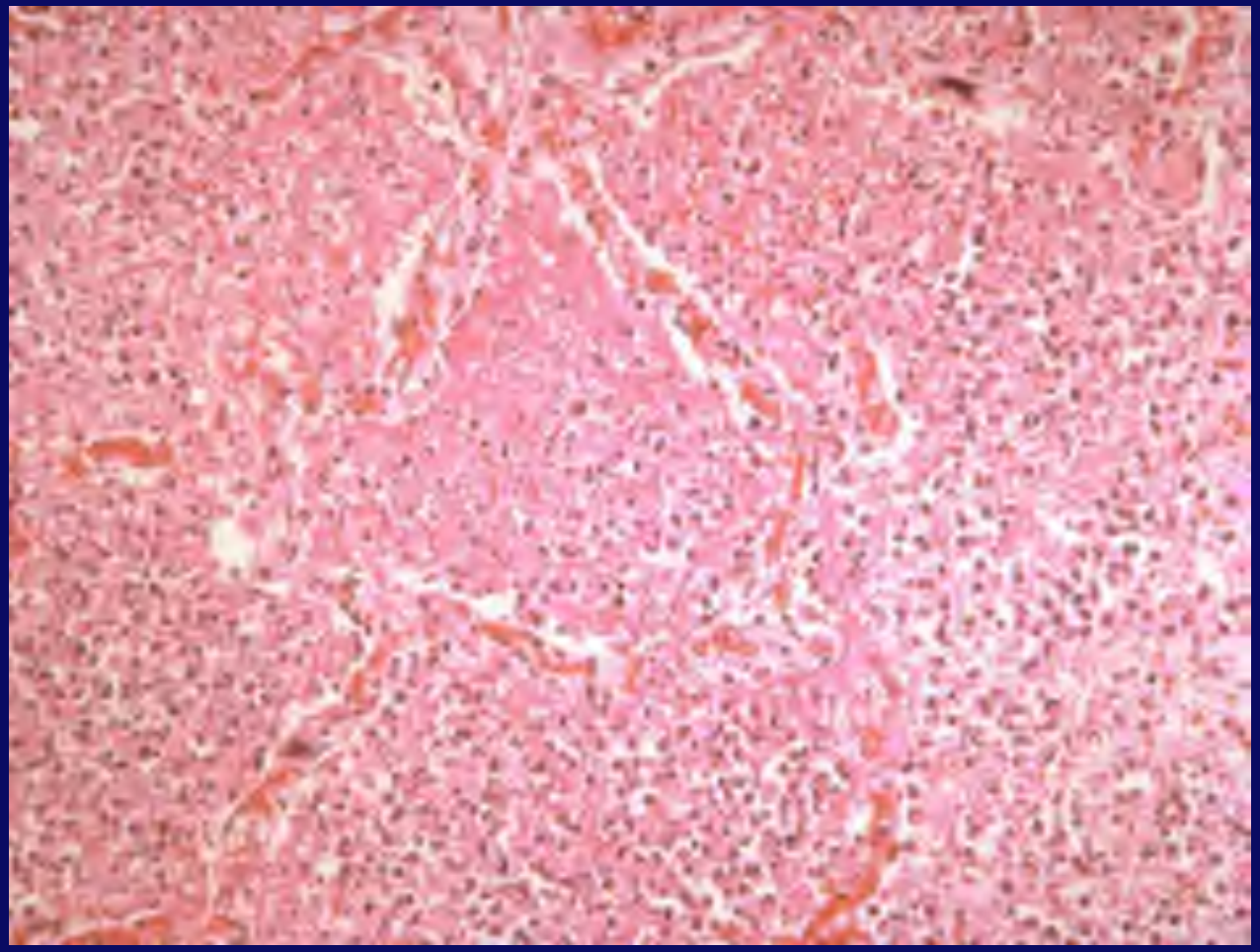
(where the first recognized outbreak of *Legionella* infection occurred)

PD-GOV Jack E. Boucher, Historic American Buildings Survey

Scope and consequences of the 1976 Bellevue-Stratford outbreak

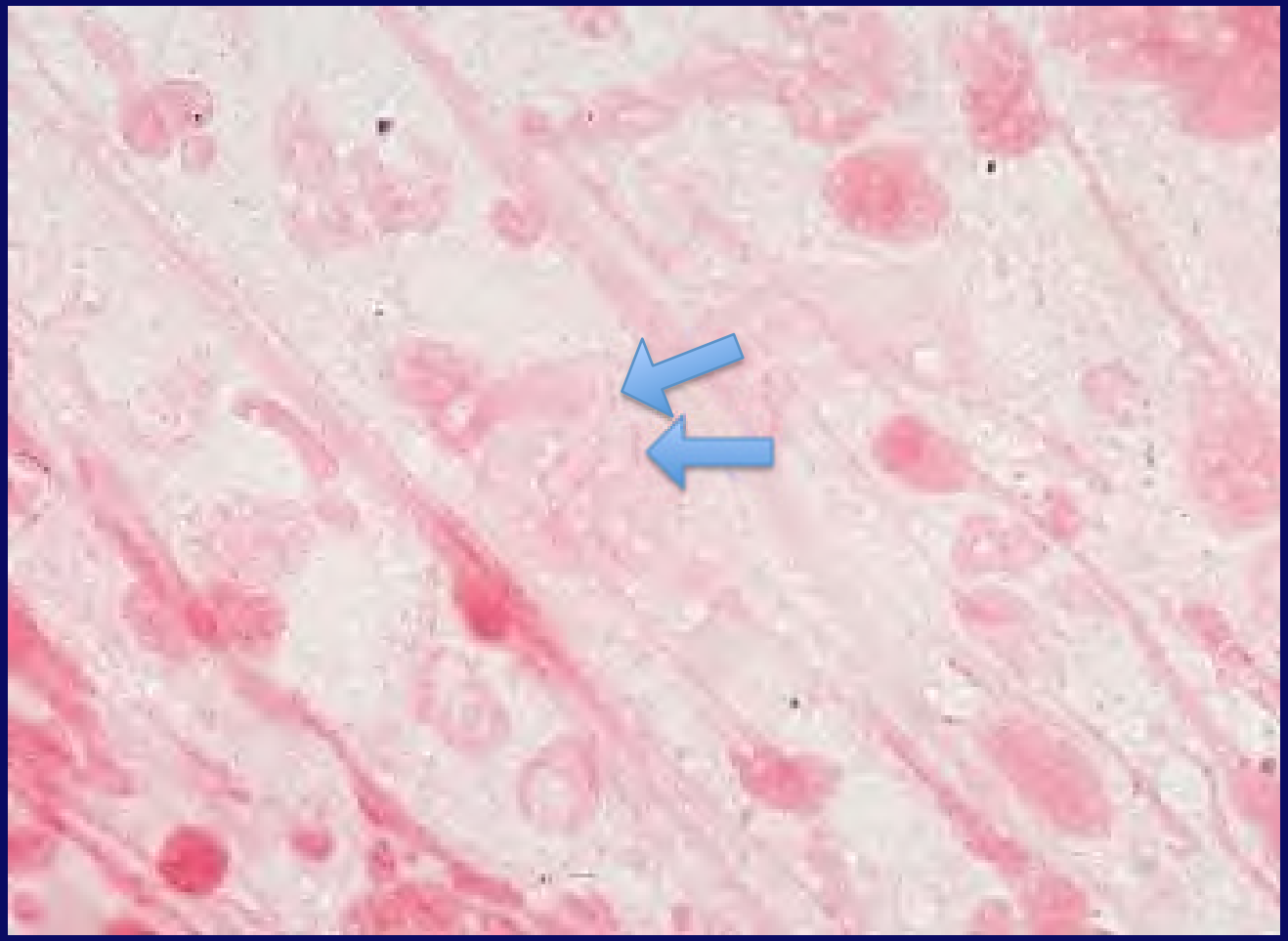
- 182 American Legionnaires become ill
- 146 were hospitalized
- 29 died (associated with respiratory failure)
- There were no secondary cases

Fibrinopurulent pneumonia



PD-INEL Source undetermined

Legionella: difficult to distinguish from the background

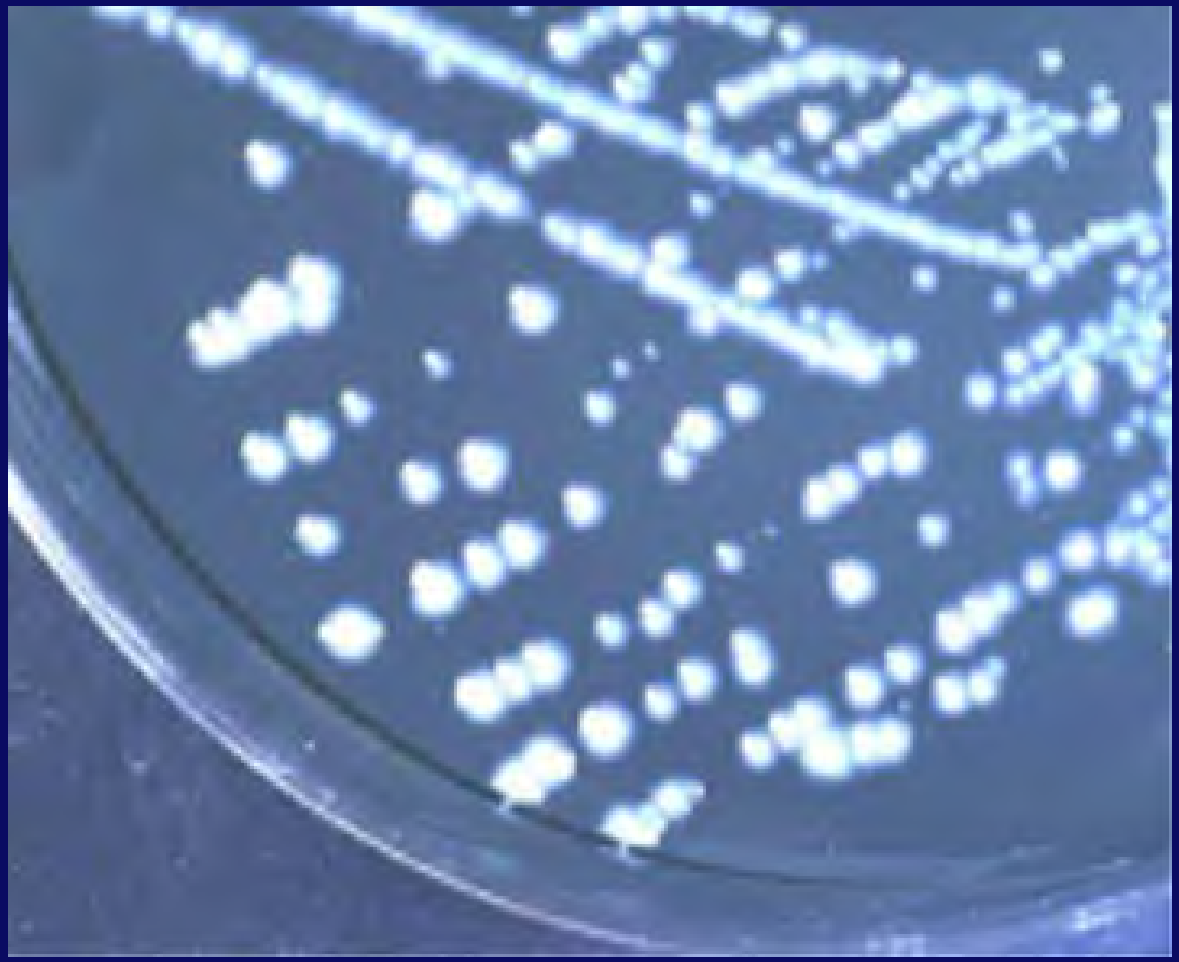


PD-INEL Source undetermined

Laboratory investigation

- Etiologic agent was unknown for months!
- Eventually, the infection was passed from the lung tissues of deceased patients to guinea pigs.
- Then, from guinea pigs to highly-enriched liquid media
- Then, a specialized agar medium

Buffered charcoal yeast extract agar



PD-INEL

Source undetermined

Supplemented with cysteine and iron pyrophosphate

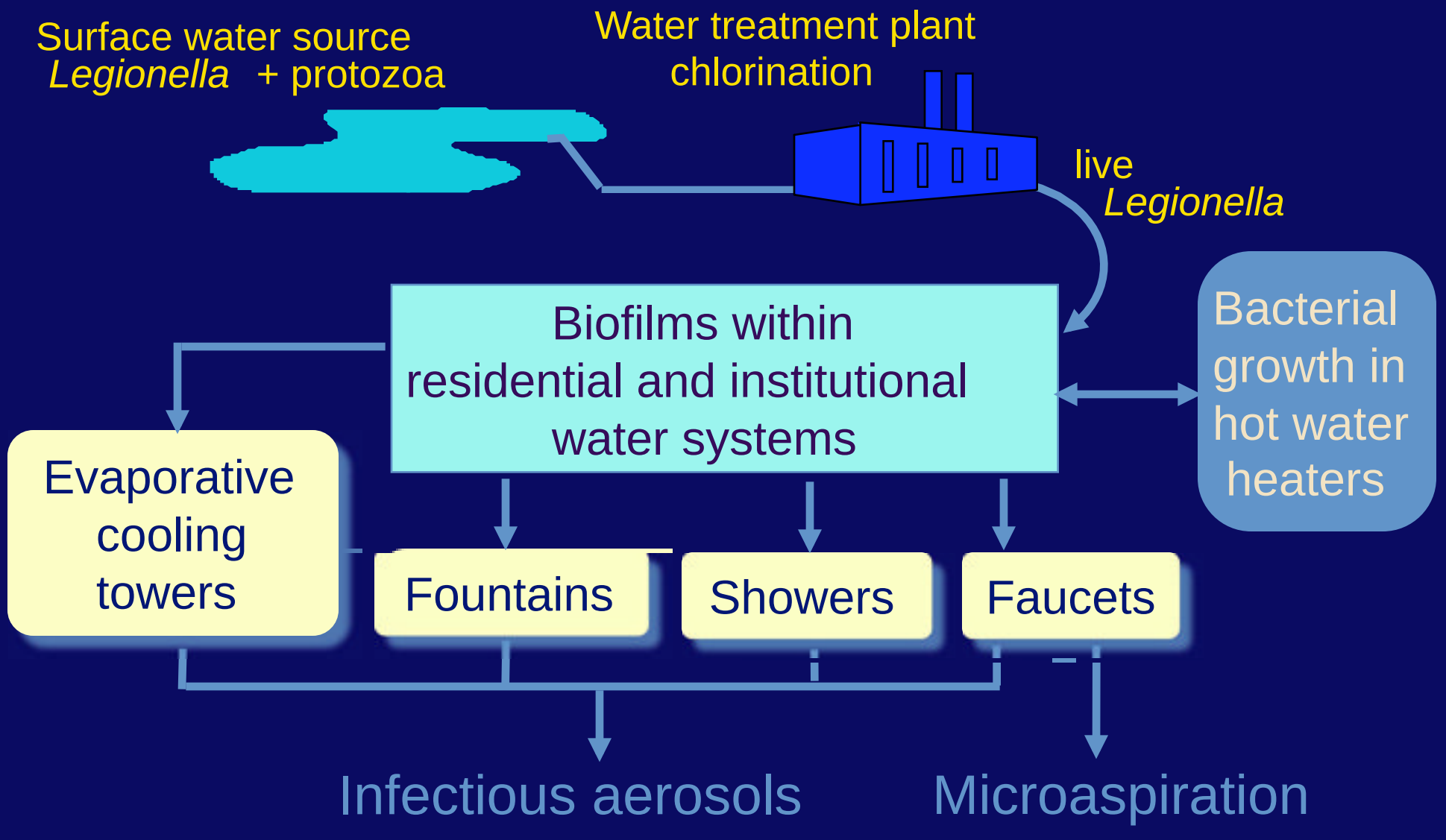
Outbreaks of legionellosis that preceded the 1976 Philadelphia outbreak

LOCATION	Year	n	Est. attack rate	Case-fatality rate
St. Elizabeth's Hosp., D.C.	1965	81	1.4%	17%
Health Dept., Pontiac, Michigan	1968	144	95%	0
James River, Virginia	1973	10	100%	0
Benidorm, Spain	1973	89	--	3.4%
Odd Fellow's Conv., Phila. PA	1974	11	2.9%	10%
American Legion Convention, Philadelphia, PA	1976	182	4.0%	17%

Identification and taxonomy

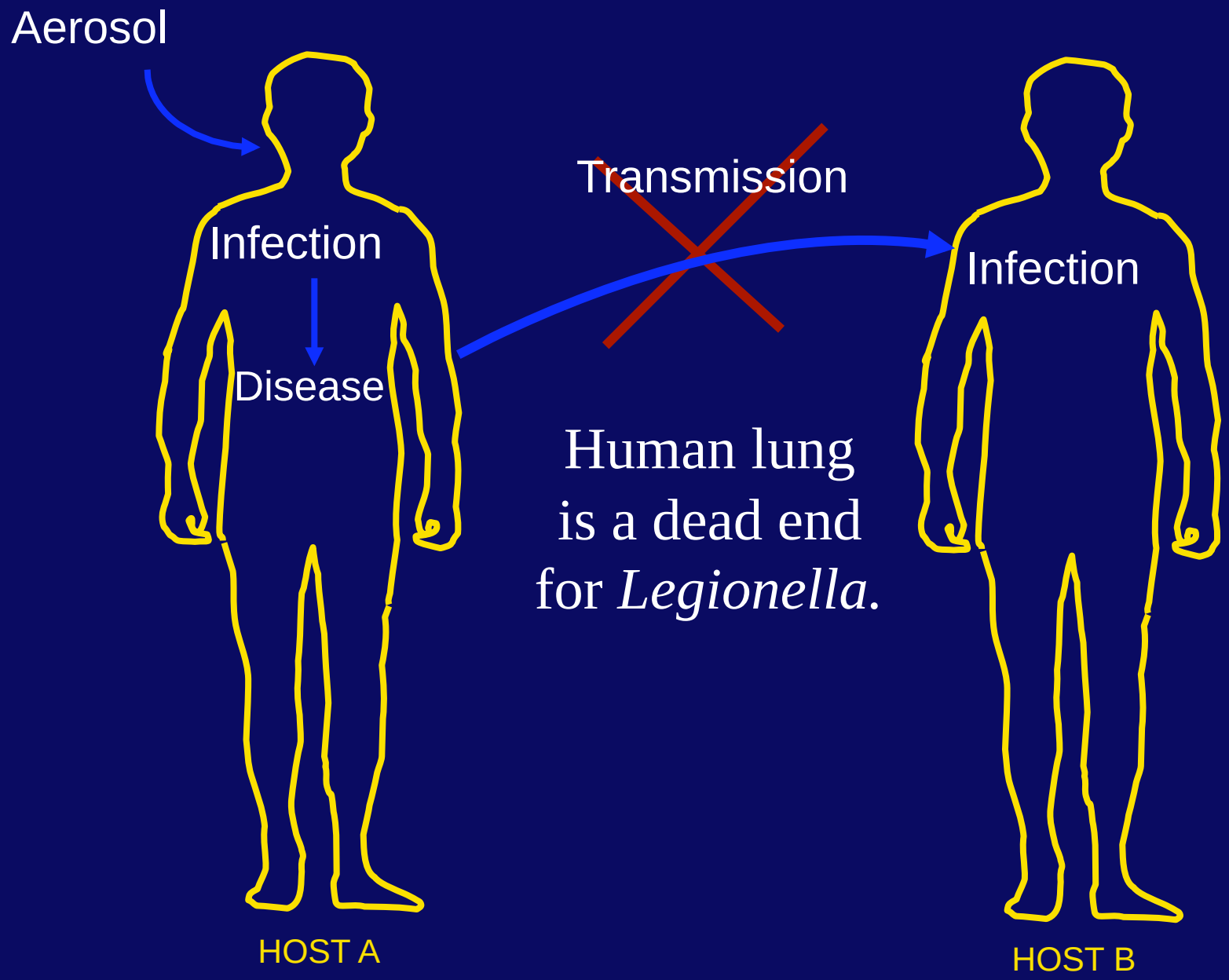
- ~50 *Legionella* spp. (19 have caused human disease)
- *L. pneumophila* causes most of human disease
  - 16 distinct serogroups
  - Most disease due to serogroup 1 (SG1) - ~60%
- Features:
  - All are flagellate GNRs, catalase-positive
  - Survive major temperature extremes (up to 55°C)
  - Identified with group-specific antisera
- LLAPs (cultivable only in amoebae)

Aqueous sources of Legionellae

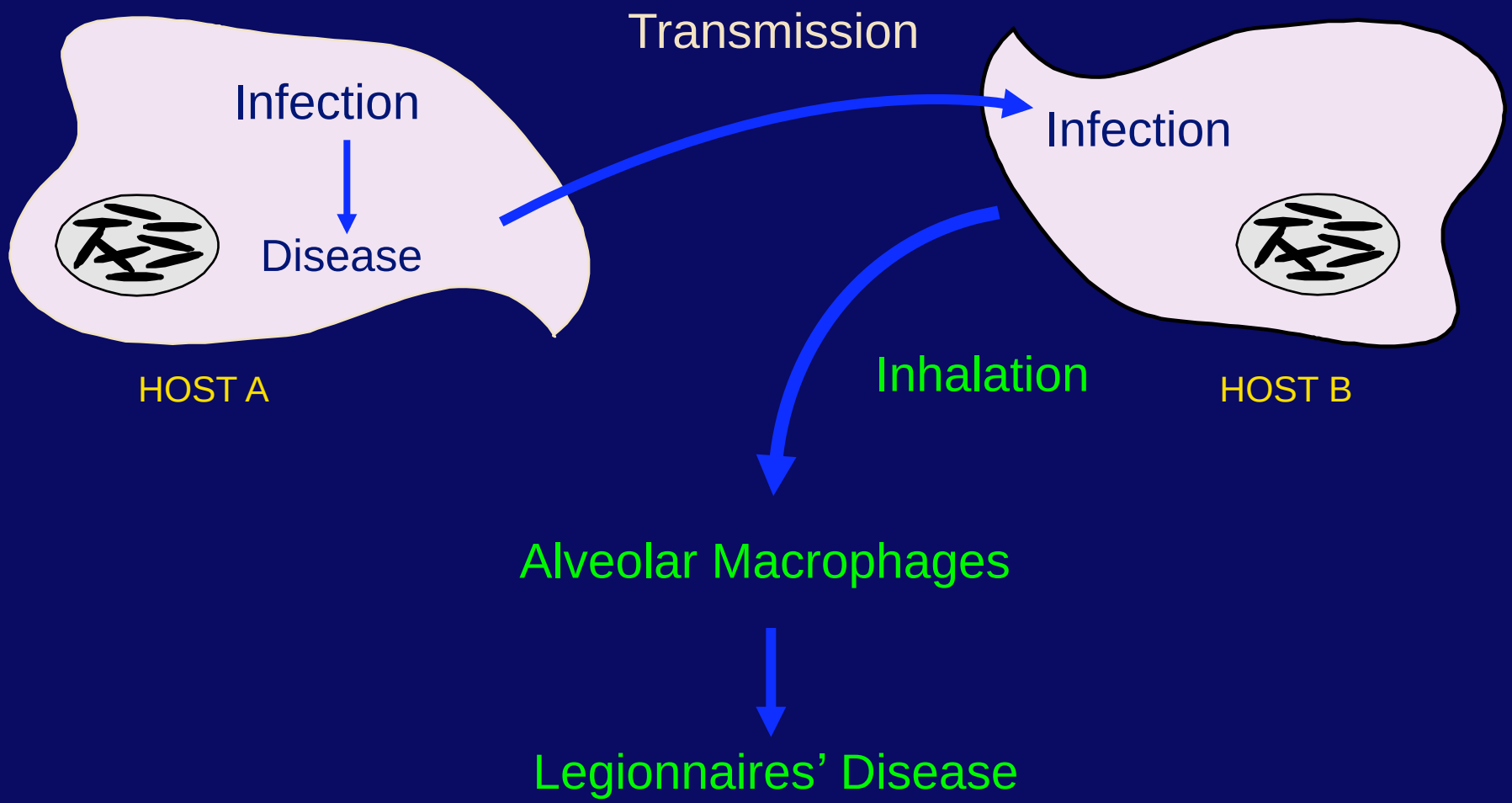


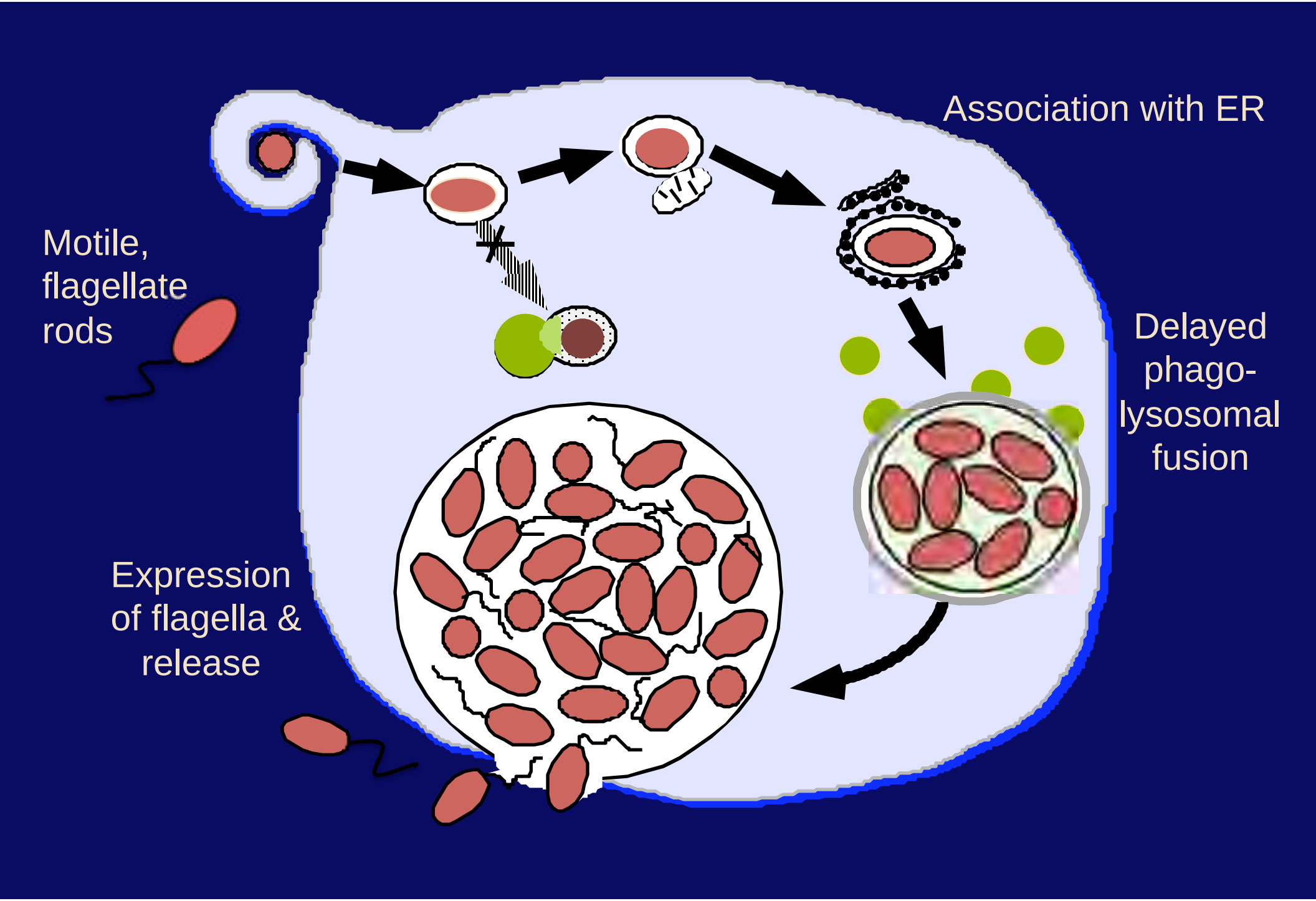
Evidence that Intracellular Infection is Essential for Legionnaires' Disease

- From animal models
  - Max. growth ~ bacteria associated with cells.
  - Susceptibility of an animal species ~ susceptibility of its macrophages to infection *in vitro*.
  - Mutants with poor macrophage growth ~ avirulent.
- From human infection
  - Intracellular bacteria are seen in lung sections.
  - Antibiotic efficacy ~ penetration of agent into cells.

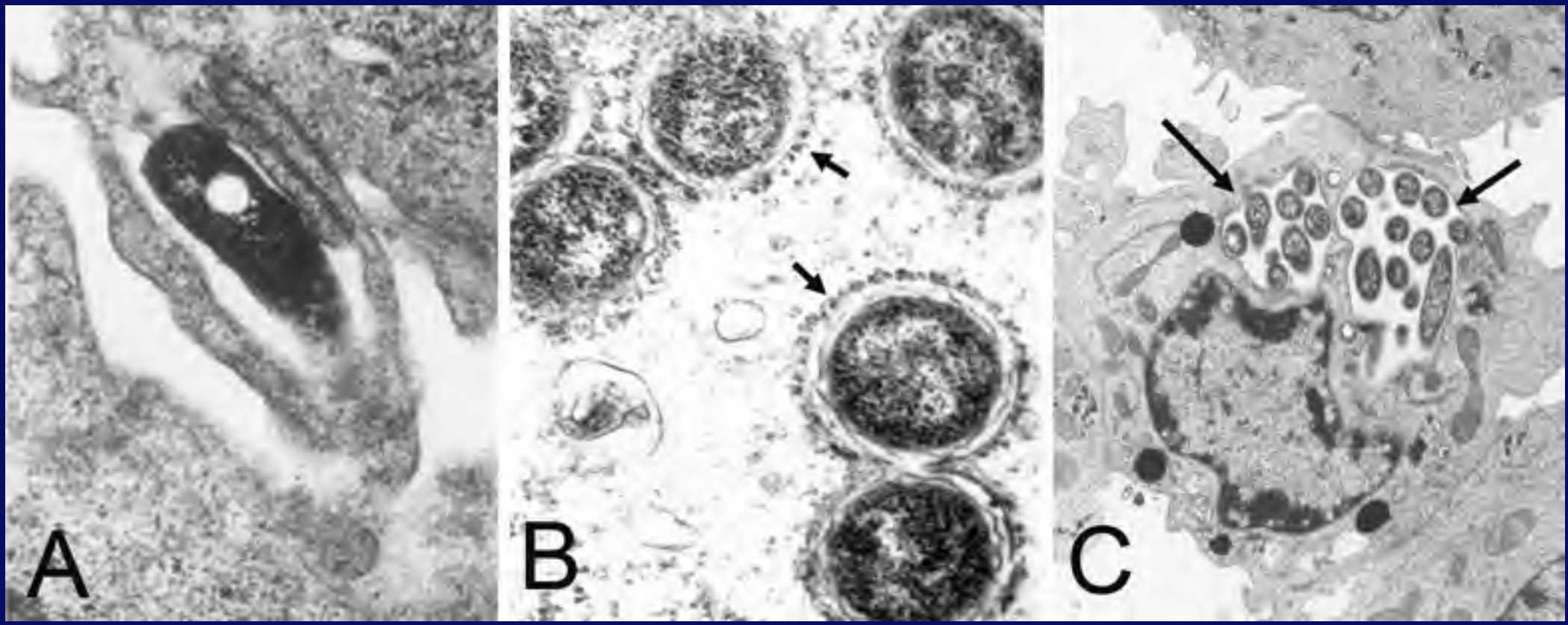

Aerosol
Infection
Disease
Transmission
Infection
Human lung
is a dead end
for *Legionella*.
HOST A
HOST B

Protozoa are the natural hosts for *Legionella*.




Association with ER
Motile, flagellate rods
Delayed phago-lysosomal fusion
Expression of flagella & release

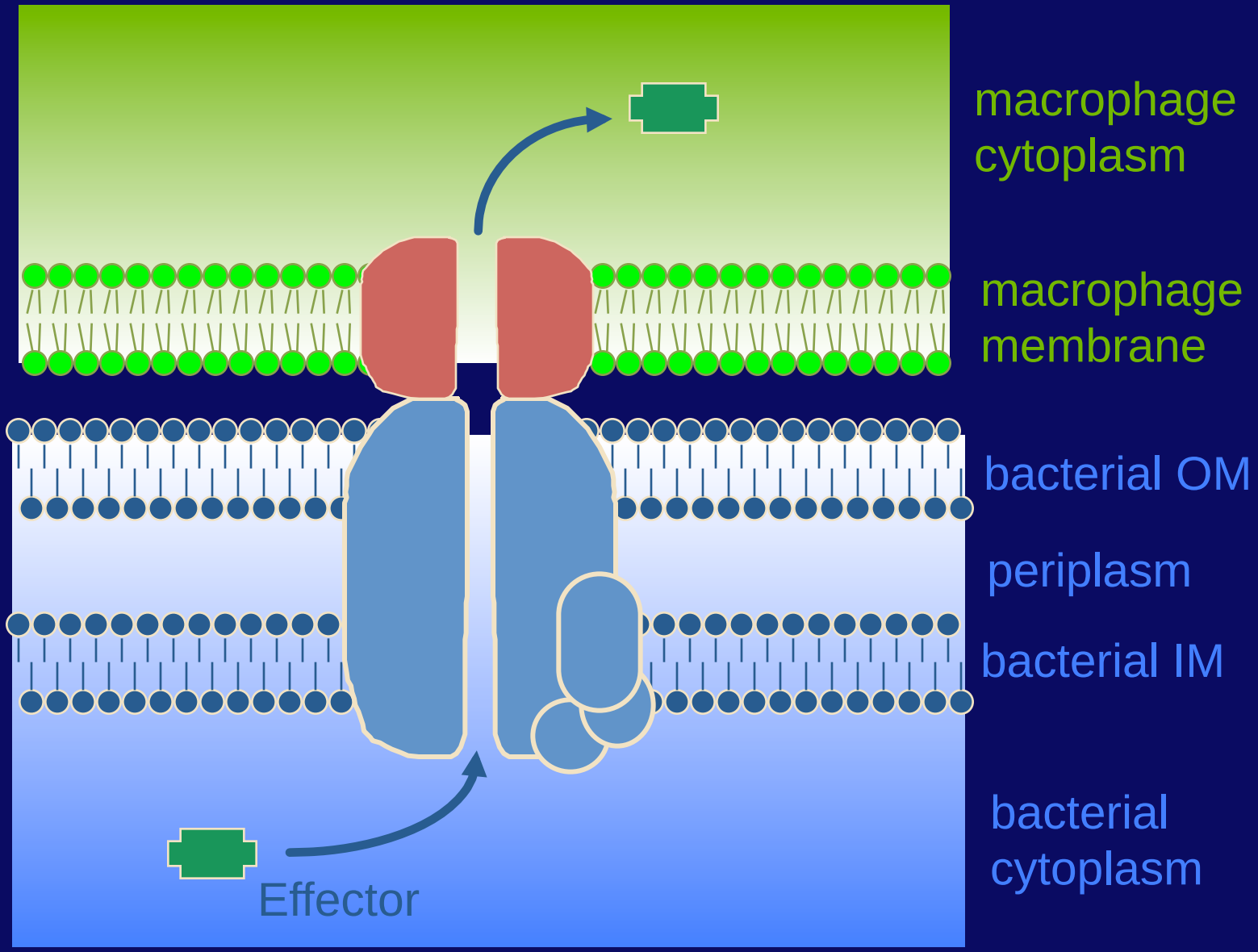
Legionella EMs

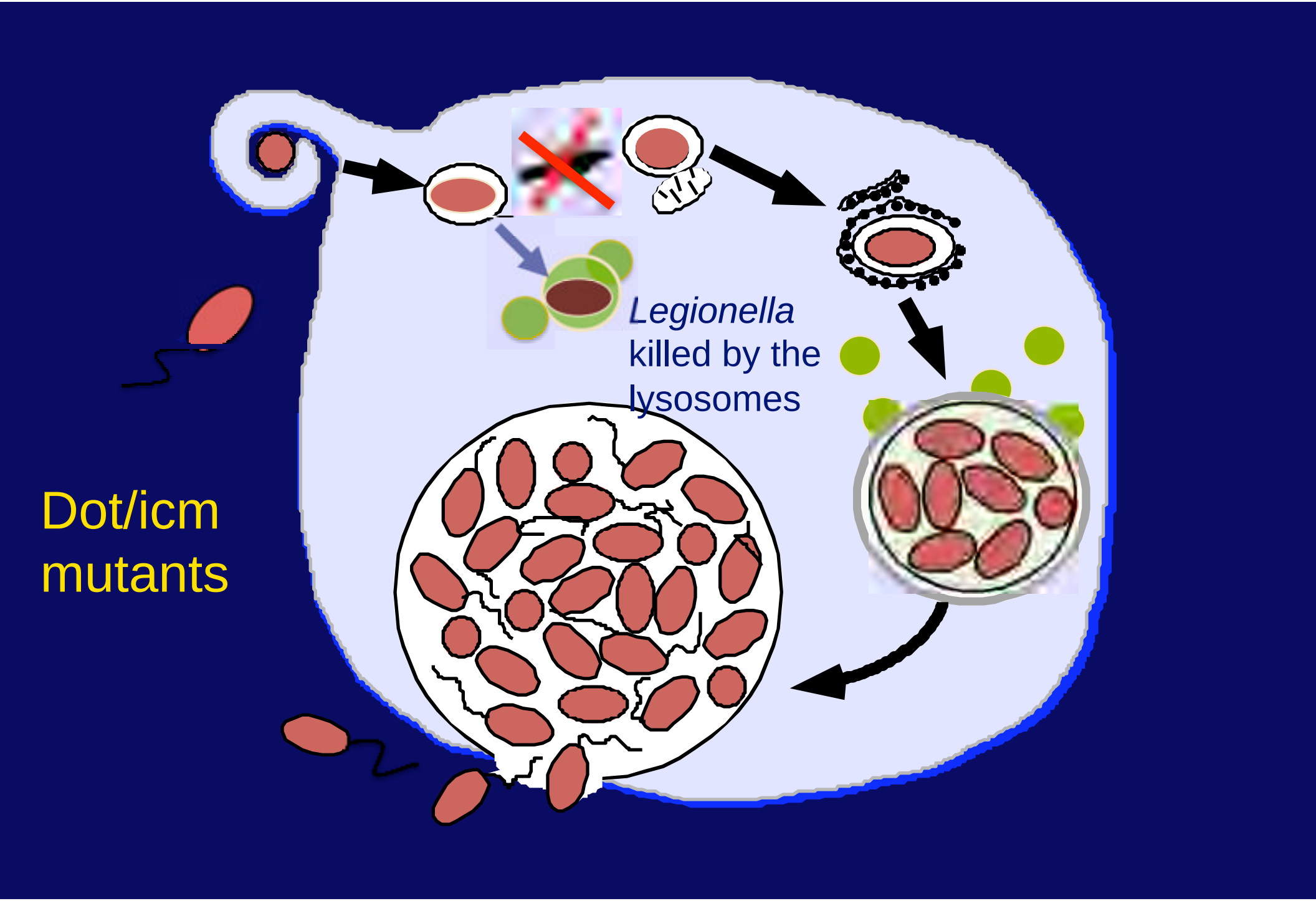


PD-INEL

Source undetermined

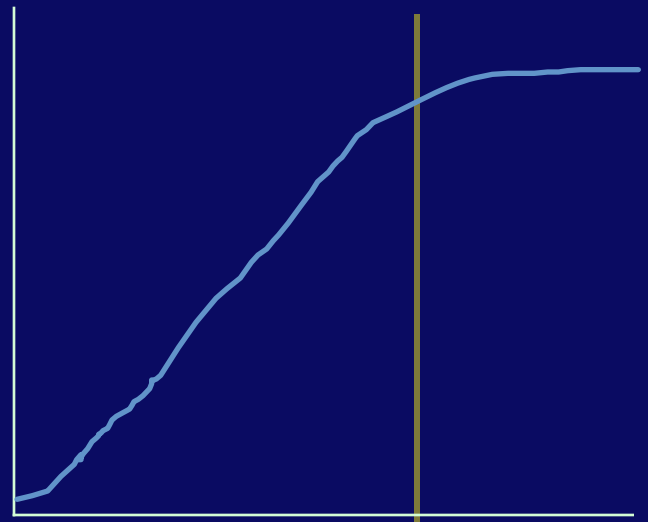
The Dot/Icm Complex




Legionella
killed by the
lysosomes
Dot/icm
mutants

Growth-phase regulation of virulence

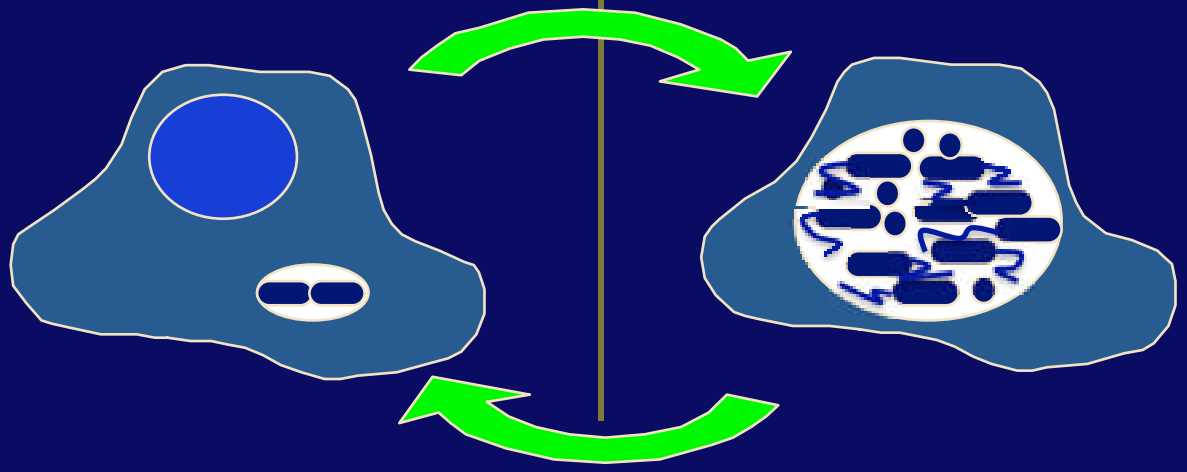
In vitro

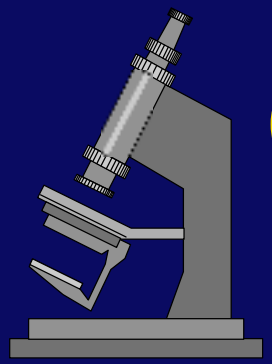


Exponential
"REPLICATIVE"

Postexponential
"TRANSMISSIVE"

In vivo





Clinical diagnostic methods

		Sensitivity	*Specificity*
Culture:	sputum (with acid)	20-80%	100%
	BAL	80-90%	100%
	lung tissue	90-99%	100%
DFA		25-75%	95-99%
Serology SG1:	seroconversion	70-80%	95-99%
	single titer > 256	10%	50-70%
Urine Ag-RIA*	(3 to >300 days)	80-98% (SG1)	99%

*accurately diagnoses only SG1 = 60% of cases

Mortality from Legionnaires' disease in 3 outbreaks, by antibiotic therapy*

	Philadelphia 1976	Vermont 1977	Wadsworth, CA 1978
Cephalosporins	46%	17%	
Aminoglycosides	36%	19%	25% (80%)§
Penicillins	23%	16%	
Erythromycin	11%	4%	7% (24%)

* Tsai *et al*. Ann Intern Med 1979; 90: 509
Broome *et al*. Ann Intern Med 1979; 90: 573
Kirby *et al.* Medicine 1980; 59:188

§ parentheses indicate immunosuppressed patients

Uptake of Antibiotics by Pulmonary Alveolar Macrophages

taken from Johnson et al. J. Lab Clin Med 1980; 95: 429-39

ANTIBIOTIC	*C:E ratio at 120 min*
Erythromycin	20.6 ± 3.1
Chloramphenicol	2.1 ± 0.2
Rifampin	1.8 ± 0.3
Tetracycline	0.9 ± 0.1
Gentamicin	0.6 ± 0.1
Cefazolin	0.07 ± 0.06

Antibiotic rx of Legionnaires' Disease

LONGSTANDING CHOICE:

High-dose Erythromycin + Rifampin

TRADITIONAL ALTERNATIVE:

Doxycycline

BETTER ALTERNATIVES:

Azithromycin and newer macrolides

Fluoroquinolones

Questions to consider

- Was the hot tub the source of the illness? Why?
- Why were none of the patient's family members or co-workers affected?
- Why was the sputum Gram stain negative?
- Why was cefuroxime ineffective?
- Why was the diagnosis made by a urine antigen test instead of a culture?

What should be done in Ghana?

- Frequency of this infection is not known
- Rapid diagnostic tests unlikely to be available
- Treat severe pneumonia on suspicion:
  - Patients on steroids or other immunosuppressive medications
  - Recent overnight travelers
  - People with exposure to heated water or water aerosols
- Therapy includes azithromycin, a fluoroquinolone, or erythromycin + rifampin

Additional Source Information

for more information see: http://open.umich.edu/wiki/CitationPolicy

Slide 6: M. Rein, CDC/Public Health Image Library, Clue cells, #3719, http://phil.cdc.gov/phil

Slide 7: Gram stain of a clue cells, source undetermined

Slide 17: Juvenile Periodontitis, http://www.erste-zahnartzmeinung.de/zahnwissen-kzvwl/zahnwissen/pa_klass.htm

Slide 21: X-ray, source undetermined

Slide 23: X-ray, source undetermined

Slide 26: Jack E. Boucher, “Belleview-Stratford Hotel,” Wikipedia Commons, http://commons.wikipedia.org/wiki/File:BelleviewStratford.jpg

Slide 28: Fibrinopurulent pneumonia, source undetermined

Slide 29: Legionella Gram stain, source undetermined

Slide 31: source undetermined

Slide 40: source undetermined