Legionella: Then and Now
Development of Knowledge About Legionella

- The first known outbreak
- Discovery of the causative agent
- Development of a medium to grow *Legionella*
- Knowledge of the reservoir and transmission of the bacterium
- Realization that outbreaks still occur
- Preventive measures?
Historical Significance

Legionella: Molecular Biology (2008)

http://www.q-net.net.au/~legion/LegionnairesDisease_Worlds_First-Outbreak.htm
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Present</th>
<th>Absent</th>
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<td>97</td>
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<td>Malaise</td>
<td>86</td>
<td>11</td>
<td>89</td>
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<tr>
<td>Cough</td>
<td>96</td>
<td>16</td>
<td>86</td>
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<tr>
<td>Chills</td>
<td>70</td>
<td>25</td>
<td>74</td>
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<td>Dyspnea</td>
<td>50</td>
<td>35</td>
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<tr>
<td>Myalgias</td>
<td>47</td>
<td>38</td>
<td>55</td>
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<tr>
<td>Headache</td>
<td>49</td>
<td>44</td>
<td>53</td>
</tr>
<tr>
<td>Chest pain</td>
<td>46</td>
<td>42</td>
<td>52</td>
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<tr>
<td>Sputum production</td>
<td>47</td>
<td>47</td>
<td>50</td>
</tr>
<tr>
<td>Purulent sputum</td>
<td>23</td>
<td>24</td>
<td>49</td>
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</tbody>
</table>
Legionnaires Disease Scare Is a Massive Hoax

By JOHN BLACKBURN and THOMAS KUNCL

The great scare over Legionnaires Disease is nothing more than a massive hoax by medical bureaucrats.

Top doctors say a "climate of fear" has been created by the government's Center for Disease Control (CDC). They charge the CDC fed scare stories to the press, leading the American public to believe a dread killer disease was sweeping the country.

And instead of trying to calm the nation, CDC director Dr. William Foege made things worse on November 9 by publicly announcing that Legionnaires Disease may strike up to 45,000 Americans a year — and kill as many as 6,000 annually.

The truth is, that CDC's scare stories are plain bunk, according to top medical experts contacted during an ENQUIRER investigation. They say Legionnaires Disease is only a minor form of pneumonia that can't even be transmitted from person to person.

Even the CDC's own expert who found the alleged "bug" that causes the disease admitted to The ENQUIRER that about 750 people a year would die from the disease — not 6,000. "CDC allowed a climate of fear to build, to magnify their own importance. That was disingenuous and they should have stopped it," declared Dr. J. Anthony Morris, formerly a top virologist with the U.S. Food and Drug Administration (FDA).

"They are making something out of nothing. It's like the swine flu scare all over again — nothing more than a scientific hoax.

"Dr. Morris blamed the CDC for hysteria headlines that leaked into newspapers, leading readers to believe that Legionnaires Disease was breaking out all over.

"The director of CDC should have been saying that what was really happening was that among hundreds of cases of pneumonia-like illnesses, there are no more cases of pneumonia associated with Legionnaires Disease," Dr. Morris said.

Dr. Hubert Ratner, former public health director of Oak Park, Ill., and former associate clinical professor of public health at Loyola University Stritch School of Medicine, agreed:

"There has been a climate of hysteria across the country — created by the CDC. You could not even pick up your newspaper without reading about this so-called dread disease. "CDC is exaggerating the importance of the cases, trying to say something is 'dangerous' or 'lethal' when it's actually nothing.

"There are dozens and dozens of different agents causing pneumonia. And the bug that CDC claims causes Legionnaires Disease — if it exists — is no worse than the others and probably milder than most. But they're misleading the public, trying to make it sound as important as it they've found the cure for cancer.

"All of those cases that have been frightening Americans were, I believe, designed by the CDC for just that purpose — to frighten us," Dr. Ratner charged, adding that it was done to get "bigger and better funding."

Critics charge that CDC exaggerates the dangers to make the public think of the bug that causes Legionnaires Disease as "more important than it was."

"CDC has blown this thing out of all proportion in an attempt to boost its image, which was badly tarnished by its handling of the swine flu scare," charged Dr. Morris, who was fired by the FDA for boasting mass inoculations for swine flu.

"The CDC is playing its own public relations game."

HEADLINES More about the disease — but doctors say government officials fed scare stories to the press. 350 confirmed cases of the disease in the U.S. since July 1976.

Dr. McEady admitted to The ENQUIRER that he is not 100 percent sure that the bacteria he discovered is the cause of the disease. The only sure way to determine if it is the cause, he added, is to deliberately infect someone with it, "and you just can't do that."

Dr. Ernest C. Herrmann Jr., microbiologist at the Univ. of Illinois School of Medicine in Peoria, blasted the CDC for failing to inform hospitals throughout the U.S. on how to identify the bacteria that causes Legionnaires Disease, and how to treat it.

In order to get a diagnosis of the disease, doctors must send blood samples to CDC headquarters in Atlanta.

"What the hell kind of medical practice is this?" Dr. Herrmann stormed angrily. "In my view, every hospital that has a bacteriology lab should be alerted as to how to grow this organism, how to identify it as fast as possible and how to treat patients properly. CDC hasn't done this."

A CDC spokesman, Dr. Walter Dowdle, did the test was still "too complex" to be duplicated in hospital labs.

But Dr. Herrmann declared bluntly: "CDC is playing its own public relations game."

Link Legion Fever & New Germ

New Legionnaires' Cases Probed 16 in Vermont
Top Doctors Claim ‘Climate of Fear’ Created by Govt.

LEGIONNAIRES DISEASE
- THE GREAT HOAX

References on *Legionella* Media and Recovery

- Others
BCYE agar:
Charcoal 2.0 g
YE 10.0 g
ACES buffer 10.0 g
Ferric Pyrophosphate 0.25 g
L-Cysteine 0.40 g
Agar 17.0 g
Potassium alpha-ketoglutarate 1.0 g
Dist. Water 1L
Yeast Extract

GPAV:
Glycine 0.3%
Polylymyxin B 100U/ml
Vancomycin 5ug/ml
Anisomycin 80 ug/ml

1 = BCYE alpha; 2=PAV; 3=GPAV; 4=PAV
The Genus *Legionella*

- >48 species, 70 serogroups
- Small, G- bacilli
- Aquatic
- require L-cysteine and soluble iron
- Facultative intracellular pathogens
- Motile
- Energy source = amino acids
- Aerobic, capnophilic
- high [phospholipid] in outer membrane
Filtering and Air Sampling for *Legionella*

Figure 1. Nucleopore filter funnel assembly for concentrating water samples.

Figure 4. An All-Glass Impinger (AGI) (foreground). An AGI properly connected to a flowmeter and vacuum pump (background).

Figure 5. A 2-stage (foreground) and a 6-stage Andersen sampler. A 6-stage Andersen sampler properly connected to a flowmeter and a vacuum pump (background).
Capillary pore membrane 10,000X Tortuous pore membrane

Cross-sectional comparison

~150 μm

~10 μm
IFA and DFA Tests

IFA:
4-fold rise in serum titer

DFA

Pontiac Fever

- Influenza like illness
- Incubation period: 1-2 days
- Symptoms: fever, chills, muscle pain, lethargy, and headache
- Symptoms will develop over a 12 hour time period and persist for 2-5 days
  - Will resolve without antibiotics

Legionnaires’ Disease

- Usually causes pneumonia
- Severe multisystem disease
  - GI, CNS, liver and kidneys
- Incubation period: 2-10 days
- Symptoms: fever, chills, dry and non-productive cough and headache.
  - Multi-organ disease is common
- Requires antibiotics
- Prevalent in late summer or autumn
Acceptable Lab Tests for LD

- Culture
- *DFA on biopsy or secretion
- 4-fold rise in antibody titer
- **Urine Antigen
Some Outbreaks Caused by *Legionella* Before 1976

<table>
<thead>
<tr>
<th>Outbreaks</th>
<th>Year</th>
<th>Disease</th>
<th>Cases/Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Elizabeth's Hospital</td>
<td>1965</td>
<td>Pneumonia</td>
<td>81/14</td>
</tr>
<tr>
<td>Washington, D. C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hotel, Benidorm, Spain</td>
<td>1973</td>
<td></td>
<td>86/3</td>
</tr>
<tr>
<td>Hotel, Philadelphia, Pa.</td>
<td>1974</td>
<td></td>
<td>20/2</td>
</tr>
<tr>
<td>Health Dept. Building, Pontiac,</td>
<td>1969</td>
<td>Pontiac</td>
<td>144/0</td>
</tr>
<tr>
<td>Michigan</td>
<td></td>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td>James River Plant, River, Virginia</td>
<td>1973</td>
<td></td>
<td>10/0</td>
</tr>
</tbody>
</table>
Where is *Legionella*?

- Lakes, rivers, streams, etc.
- Cooling towers
- Hot tubs
- Decorative fountains
- Plumbing systems
- Evaporative Coolers
- Humidifiers
- Other
# Examples of Epidemic Sources for Legionella

<table>
<thead>
<tr>
<th>Outbreak Site</th>
<th>Year</th>
<th>Legionella Source</th>
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</thead>
<tbody>
<tr>
<td>Hospital, Providence, R. I.</td>
<td>1983</td>
<td>Cooling tower</td>
</tr>
<tr>
<td>*Bank Building, N. Y., N. Y.</td>
<td>1984</td>
<td>Cooling tower</td>
</tr>
<tr>
<td>Country Club, Atlanta, Ga.</td>
<td>1978</td>
<td>Evaporative condenser</td>
</tr>
<tr>
<td>Hospital, Paris, France</td>
<td>1981</td>
<td>Potable water</td>
</tr>
<tr>
<td>*Spa, Vermont</td>
<td>1983</td>
<td>Whirlpool</td>
</tr>
<tr>
<td>Hospital, England</td>
<td>1981</td>
<td>Showerhead</td>
</tr>
<tr>
<td>Hospital, Chicago, Ill.</td>
<td>1982</td>
<td>Respiratory therapy equipment</td>
</tr>
</tbody>
</table>

* Pontiac Fever outbreak.
### Examples of Epidemic Sources for *Legionella*

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<th>Legionella Source</th>
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<tr>
<td>*Bank Building, N. Y., N. Y.</td>
<td>1984</td>
<td>Cooling tower</td>
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<td>1978</td>
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<td>1981</td>
<td>Potable water</td>
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<tr>
<td>*Spa, Vermont</td>
<td>1983</td>
<td>Whirlpool</td>
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<td>Hospital, England</td>
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<tr>
<td>Hospital, Chicago, Ill.</td>
<td>1982</td>
<td>Respiratory therapy equipment</td>
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* Pontiac Fever outbreak.
<table>
<thead>
<tr>
<th>DATE</th>
<th>CITY</th>
<th>SITE</th>
<th>CASES</th>
<th>PUTATIVE SOURCE</th>
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<tbody>
<tr>
<td>Nov 85</td>
<td>Wollongong</td>
<td>Club</td>
<td>8</td>
<td>Evaporative Condenser For Beer Cooler</td>
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<td>Jan 86</td>
<td>Adelaide</td>
<td>Community</td>
<td>21</td>
<td>A.C. Cooling Tower</td>
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<td>April 87</td>
<td>Wollongong</td>
<td>Shopping Centre</td>
<td>44</td>
<td>A.C. Cooling Tower</td>
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<td>Dec 88</td>
<td>Adelaide</td>
<td>Community</td>
<td>19</td>
<td>Gardening Potting Mixtures</td>
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<td>Mar 89</td>
<td>Burnie</td>
<td>City Centre</td>
<td>17</td>
<td>A.C. Cooling Tower</td>
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<td>May 89</td>
<td>Sydney</td>
<td>Club</td>
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<td>Evaporative Condenser For Beer Cooler</td>
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<td>Species</td>
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<td>Species</td>
<td>Serogroups</td>
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<td>------------</td>
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<td>L. pneumophila</td>
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<td>L. jamestowniensis</td>
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<td>L. anisa</td>
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<td>L. birminghamsensis</td>
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<td>L. longbeachae</td>
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<td>L. bozemanii</td>
<td>2</td>
<td>L. maceachernii</td>
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<td>L. micdadei</td>
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<td>L. cherri</td>
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<td>L. moravica</td>
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<td>L. cincinnatiensis</td>
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<td>L. dumoffii</td>
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<td>L. erythra</td>
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<td>L. feeleii</td>
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<td>L. gormanii</td>
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<td>L. santicrucis</td>
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<td>L. hackeliae</td>
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<td>L. spiritensis</td>
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<tr>
<td>L. israelensis</td>
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<td>L. steigerwaltii</td>
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<tr>
<td></td>
<td></td>
<td>L. wadsworthii</td>
<td>1</td>
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</table>
Life-cycle of *Legionella pneumophila* in protozoa and human macrophages

Pathogenesis
Protozoa Interactions


Sources of Monoclonal Antibodies Used in the International Panel for *Legionella pneumophila* SG1


- W32 (no. 4): Watkins, et. al., 1985, J. Hyg. 95:211


Monoclonal Ab Subtyping of *Legionella pneumophilia* serogroup 1

![Monoclonal Antibodies](image)
**International Monoclonal Antibody Panel for *Legionella pneumophila* SG1**

<table>
<thead>
<tr>
<th>Type</th>
<th>MAB1</th>
<th>MAB2</th>
<th>MAB3</th>
<th>W32</th>
<th>33G2</th>
<th>32A12</th>
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<tr>
<td>Knoxville 1</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>v</td>
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<tr>
<td>France 5811</td>
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<td>-</td>
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<td>Camperdown 1</td>
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<td>Bellingham 1</td>
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<td>+</td>
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Reactivity:
- +: Positive
- -: Negative

**Results:**
- (1,2,5,6)
- (1,2,5)
- (1,2,5,7)
- (1,2,3)
- (1,2)
- (1,6,7)
- (1,6)
- (1,3,6,7)
- (1)
- (1,4,7)
- (1,3,6)
- (-)
• Direct immunofluorescence assays

https://courses.cf.cornell.edu/edm/200/micromapcases/methods/tab.htm

Diagnosing legionellosis nucleic acid detection
• Polymerase chain reaction (PCR) assays
  – ribosomal RNA (rRNA) genes or their intergenic spacer regions
  – a gene coding for heat-shock protein (dnaJ)
Diagnosing legionellosis using nucleic acid detection

- **Benefits**
  - *Legionella* PCR could potentially detect all serogroups of *L. pneumophila*
    - useful in the early diagnosis of infections, particularly in nosocomial cases
  - PCR methods could have important economic benefits.
  - Their use in outbreaks of legionellosis could help to rapidly rule out implicated sites

- **Limitations of PCR assays**
  - Current data are insufficient for reliably estimating PCR sensitivity and specificity values, or for comparing PCR to other methods
  - Requires evaluation and standardization of sample preparation and PCR protocols
  - Define primer and probe specifications and assay sensitivities, and to reduce the effect of PCR inhibitors

Subtyping Procedures

- Serotyping
- Monoclonal antibody panel
- Ribotyping
- Multilocus alloenzyme
- PFGE
- Arbitrary Primer PCR
- Nested Primer analysis
- Sequence base typing
- Direct sequence analysis
Bogalusa Community-wide LD Outbreak

- 33 cases confirmed by IFA titer or urine antigen
- 8 deaths
- No cultures from patients
- In a town with paper mill mists
- More females than males as cases
- Case control study done by CDC and LA
Legionnaires’ Disease
Bogalusa, Louisiana

Date of hospital admission

Cases

Confirmed Cases
Possible Cases
The search for the outbreak strain.

Lab:
- 114 sputum samples; culture negative
- 2 autopsies; Lp-1 by DFA

Results:
- 2 specimens Lp-1; monoclonal subtype 1,2,5,6
Mist in Grocery's Produce Section Is Linked to Legionnaires' Disease

BY PETER APPLEBOME
Special To The New York Times

ATLANTA, Jan. 10 — Health officials said today that an automatic misting machine in a Louisiana supermarket caused a major outbreak of Legionnaires' disease recently, and supermarket chains around the country said they had stopped using similar machines as a precaution.

Officials with the Centers for Disease Control here and the Louisiana Office of Public Health said today that they had traced 34 confirmed cases of the disease, including two fatal cases, to a mist system at a Winn-Dixie supermarket in Bogalusa, La. The device keeps produce fresh by automatically releasing a fine sprays, and hundreds of them are in use around the country.

Health officials said today that they believed the outbreak was an isolated one and that there are specific features in the system in Bogalusa that may have made it more likely to spread the disease than most others in use. They emphasized that because victims contract the disease only by inhaling water vapor droplets containing the bacteria that cause the disease, there is no danger to people who eat sprayed produce.

Legionnaires' disease is a respiratory ailment that causes flu-like symptoms and can be treated with antibiotics. It is fatal in about 15 percent of the cases.

But officials said that because many cases of the disease are not reported when it is mistaken for something else, it is possible that such systems could be responsible for other Legionnaires' cases. And a leading expert on the disease at the Centers for Disease Control said that the Louisiana cases could be an important finding.

"This is the first report linking Legionnaires' disease to a mist machine," said the expert, Dr. Charles Hoge, who is not directly involved in the Bogalusa case. "It could have some important implications if that ends up being definitively shown to be cause."

A spokesman for the Food and Drug Administration said the Federal agency was considering issuing an advisory recommendation to stores urging that food stores be reminded about proper maintenance of the machines.

The Centers for Disease Control records about 500 to 1,000 cases of the disease annually, but experts believe the incidence is far higher.

Continued on Page 12, Column 1
Preventive Measures

- Cleaning and chlorination
- Don’t make aerosols
- Maintenance of cooling towers & water systems
- Maintain hot tubs (spas)
- Consideration of the engineering aspects
- Follow guidelines
- Connect surveillance and subtyping
Legionella Today

• Outbreaks still occurring
• Number of cases reported to the CDC about 15K/yr.
• Worldwide awareness
• Guidelines available
• More assistance at the county/state level
• Subtyping more advanced
• 2013 7th International meeting on *Legionella* in Melbourne, Australia