Legionnaires’ disease: Results From - and Evaluation of - a Quantitative Microbial Risk Assessment

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Background

*Legionella* and Legionnaires’ Disease

- Legionnaires’ disease (LD) - first reported in 1976, American Legion Convention, Philadelphia
- LD caused by various species of the bacterial genus Legionella and most commonly *L. pneumophila* (*Lp*)
- Community acquired pneumonia attributed to Legionella may be between 4 and 20%
- LD remains rather prevalent (10,000 to 30,000 cases estimated per year in the USA)
- LD is a pneumonia that typically has a 5 to 15% mortality rate once developed to the point that medical care is needed
Background (continued)

*Legionella* and Legionnaires’ Disease

- Widely distributed in soils and surface waters
- *Legionella* are relatively chlorine resistant, compared to usual water quality indicator bacteria
- *Legionella* thrive in warm water environments, including hot water supply systems, cooling towers, hot tubs, thermal springs, and more
- Natural hosts are protozoa. The mechanisms *Legionella* evolved to replicate in protozoa also subvert human alveolar macrophage bactericidal mechanisms
Legionnaires' Disease Progression

Probability (P) of exposure > P mild infection > P clinical disease > P mortality
Research Objectives

- Develop a Quantitative Microbial Risk Assessment (QMRA) model for LD
- Evaluate the model’s adequacy for quantitatively estimating human health risks resulting from Legionella exposure - do the binomial confidence intervals of the calculated and reported rates overlap?
- Identify strengths and limitations of the data used for this QMRA model
Information from the dose-response assessment (branch 1) and the exposure assessment (branch 2) converges at the risk assessment and validation stage (3). Data from outbreaks of Legionnaires' disease were applied to validate the risk assessment model.
Dose-Response, Animal Model Selection

Guinea pigs provide a reasonable animal model and dose-response data for human risk projections

- Similar course of the disease in guinea pigs & humans
- Similar *in vitro* uptake (and replication) of Legionella in human & guinea pig alveolar macrophages
- Most mouse and rat strains appear resistant to Legionella infection due to less compliant alveolar macrophages
- Non-human primates also show resistance, but the data are limited
Animal Dose-Response Models

Dose-response models allow the extrapolation of responses to doses below the experimental range used to set the model parameters

• Models provide the response probability at a given dose
• Model parameters were fit to Legionella dose-response data using maximum likelihood techniques
• Other models run, but exponential and approximate beta-Poisson models selected for subsequent risk projection work due to mechanistic considerations and low dose extrapolation limited by exposure probability
• Goodness of fit tested & passed for the data sets employed
Dose-Response Models (Continued)

Data for guinea pig aerosol exposure studies include mortality and sub-lethal infection:

- The mortality data were evaluated as a basis for mortality or clinical severity (e.g., pneumonia with hospitalization) disease.
- The sub-lethal infection (e.g., fever, spontaneous recovery) data were evaluated as a basis for projecting human risks of infection as measured by seroconversion in an outbreak.
- Infectivity (ID) and mortality (LD) dose-response curves in guinea pigs developed for the selected dose-response models.
- The low-dose extrapolation results were examined for the various models.
Dose-response Model Findings

- The estimated responses are, for guinea pigs, as retained dose in animal lungs:
  - LD50% 6200 CFU, LD1% 92 CFU
  - ID50% 12 CFU, ID1% 0.17 CFU
- For the exponential model
  \[ P(d) = e^{-rd} \]
  Where \( P(d) \) = probability of response and \( d = \) dose
  the best fit \( r = 0.06 \) for infectious dose and \( r = 1.07 \times 10^{-4} \) for mortality
- Dose scaling is not needed for intracellular pathogens
- No interspecies factors were applied for the guinea pig to human extrapolation
Estimates of Human Exposure in Selected Outbreaks

Estimating exposures for the outbreaks was necessary for our LD QMRA model evaluation

• No LD outbreak reports existed with relevant air concentration data (investigations emphasize resolution, not research!) and LD rates
• Cooling towers were considered for evaluation but presented several obstacles
• Whirlpool spas have been implicated in numerous LD outbreaks
• Natural Hot Spring Spas have also had numerous LD outbreaks, including several well-documented outbreaks in Japan

If direct data on outbreak exposures were available, the LD QMRA Model would be largely irrelevant
Exposure (continued)

• Three LD outbreaks - a whirlpool spa outbreak and two hot spring spas - were selected (few if any others had close to adequate data for exposure estimating)
• The data available were assembled
• Approaches for resolving unreported data needed for exposure predictions were developed
• Exposures for the outbreak cases were estimated using exposure modeling techniques, including:
  • Near field-far field dispersion for the whirlpool spa
  • Bacterial water to air partitioning coefficient for the natural hot springs
Whirlpool Spas
Whirlpool Spa Outbreak

- Closest to ideal data from West Frisian Floral Show Outbreak (The Netherlands, 1999), a large outbreak with up to 318 cases (188 confirmed and probable, remainder suspected cases) cases and 29 fatalities (21 among confirmed and probable for an 11% fatality rate)
- Most detailed data available for personnel who worked in the exhibition halls – a subset of the total cases
- Aerosol generation data for whirlpool spas (but not aerosol Legionella content) are from published sources
- Microbial enrichment in aqueous aerosol (compared to bulk water phase) can occur due to rising bubble surface scavenging of bacteria and surface film droplet formation mechanisms
Whirlpool Spa (continued)

- Infection rates versus distance from source reported by Nagelkerke 2003 for workers in Hall 3
- Clinical Legionellosis rates, work hours in Hall 3, some building physical dimension reported by den Boer 2002
- No air concentration data on Lp during or after the outbreak or water concentration data for Lp in the likely source spa are available
  - Presume water content as for whirlpool spas from other LD case reports
  - Estimate Lp “generation rate” from respirable aerosol concentrations with and without bacterial enrichment factor
- Estimate exposures via Monte Carlo simulation and near field, far field air concentration model
Example of Predicted Exposure Dose Distribution from Monte Carlo Simulation

Far field, > 15 meter distance, partial enrichment of the aerosol compared to assumed bulk water concentration of Legionella
Hot Spring Spas
Japanese Natural Hot Spring Spas Exposure Assessment

- Several reasonably documented reports exist for outbreaks from these natural hot spring spas.
- Generally no air injection as in whirlpool spas.
- Data from a swimming pool investigation and the ratio of water to air endotoxin levels were used to estimate the microbial water to air partitioning coefficient and then an aerosol concentration from the reported water concentrations of Legionella.
- More direct scenario and fewer assumptions needed to estimate exposures than were used for the West Frisian whirlpool estimates.
Japanese Natural Hot Spring Spas (continued)

- Exposure time (typically 15 minutes) from an estimate given in personal communication from an industrial hygiene colleague in Japan
- Only clinical infection and mortality rates were reported
- Estimated sub-clinical infection rates from Nagelkerke (West Frisian Floral Show outbreak) ratio of clinical to sub-clinical rates
## Summary of Estimated Exposures & LD Risks

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>Estimated Dose (MEAN)</th>
<th>Subclinical Severity Infection</th>
<th>Clinical Severity Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whirlpool Spa</td>
<td>10 CFU</td>
<td>3.9×10⁻¹</td>
<td>8.9×10⁻⁴</td>
</tr>
<tr>
<td>Hot Springs Spa 1</td>
<td>47 CFU</td>
<td>9.1×10⁻¹</td>
<td>4.1×10⁻³</td>
</tr>
<tr>
<td>Hot Springs Spa 2</td>
<td>2.3 CFU</td>
<td>1.3×10⁻¹</td>
<td>2.0×10⁻⁴</td>
</tr>
</tbody>
</table>
## Summary of the Evaluation of the Calculated versus Reported Risks

(Green check = overlap of confidence intervals)

<table>
<thead>
<tr>
<th>LD Outbreak</th>
<th>Subclinical Severity Infection</th>
<th>Clinical Severity Infection</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Frisian &lt;15 meter group</td>
<td>✓</td>
<td>Reported &lt; 10 X higher</td>
<td>✓ (a)</td>
</tr>
<tr>
<td>West Frisian &gt;15 meter group</td>
<td>✓</td>
<td>Reported &lt; 10 X higher</td>
<td>✓ (a)</td>
</tr>
<tr>
<td>Miyazaki</td>
<td>“Reported” &lt; 10 X lower (a)</td>
<td>✓</td>
<td>Reported &lt; 10 X lower</td>
</tr>
<tr>
<td>Shizuoka</td>
<td>“Reported” &lt; 10 X lower (a)</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

(a) Estimated since mortality did not occur in the West Frisian worker groups and subclinical infection data were not reported for the hot springs spa outbreaks.
Summary of Key Assumptions

- Equivalent virulence of Legionella strains in the animal and human data

- For the West Frisian outbreak
  - Concentration of Legionella in whirlpool spa
  - Ventilation rate and mixing height
  - Constant emission rate of Legionella-containing aerosol

- For the hot spring spa outbreaks
  - All visitors were exposed
  - Visitors had same exposure duration
  - The endotoxin based partitioning coefficient from a swimming pool holds for whole bacteria in a hot spring spa environment
Guinea pig mortality data predicts infection rates slightly less than the reported human clinical infection rates and in general better matches the reported human mortality rates.

For the outbreaks used, the reported rates of disease span several orders of magnitude.
- The estimated exposures and the calculated rates of disease also span several orders of magnitude.
- The QMRA model predictions generally held over that several order of magnitude range.

The guinea pig data were almost all from exposure to *L. pneumophila* SG1. The outbreaks used for model evaluation also involved *Lp* SG1. Thus the QMRA may not extrapolate to other Legionella species and strains.
Some Research Implications

• QMRA appears to work for an aerosol exposure route pathogen
• Comparative immunology is important in appropriate animal model selection in QMRA
• The focus on finding only high level sources in Legionella outbreaks many be an incomplete strategy. Widely dispersed exposure sources with somewhat elevated Legionella content may need more consideration
• The Legionella QMRA results could be a basis for deriving risk-based limits on cooling towers, whirlpools, hot springs spas, and other potential Legionella exposure sources
A Few Research Opportunities -

• Develop stronger data for microbial water to air partitioning
  • Whirlpool spas
  • Natural hot springs
  • Cooling towers

• Develop more information on *in vitro* alveolar macrophage comparative immunology to better guide animal model choices for intracellular pulmonary pathogens

• Evaluate *in vitro* alveolar macrophage studies’ capability to provide new data on low-dose pathogen exposure. For Legionella, does the initial virulence state or density affect
  • Lag time/incubation period,
  • Survival fraction
  • Likelihood of eventual progression to a wide-spread infection

• Include consideration of human susceptibility factors in the QMRA framework
For Further Information

