**COXIELLA BURNETII**

**General characteristic/background**

- *Coxiella burnetii* is a gram-negative bacteria with two growth phases (the virulent phase I and the avirulent phase II), as well as a spore form.\(^1\) Phase I form is responsible for Q fever, while the phase II form does not cause disease in immunocompetent animals.\(^2\)
- Size: 0.2-0.4μm wide, 0.4-1μm long \(^3\)
- The bacteria is resistant to heat, drying and many common disinfectants, enabling them to survive for long periods in the environment.\(^1\)
- The infectious dose of *Coxiella burnetii* for human is 1-10 organisms.\(^1\)
- Because of its resistance to environment and the low airborne infection dose required, *Coxiella burnetii* is cataloged as a class B select agent by the CDC.
- Susceptible to tetracycline’s, doxycycline, and a combination of erythromycin and rifampin \(^1\)
- *Coxiella burnetii* was named after Herald Ree Cox and Macfarlane Burnet, who contributed to identifying the organism as the cause for Q fever. \(^1\)

**Bacteria Transmission**

- Transmitted to animals by infested ticks and through aerosols formed from fluids of infected animals. \(^4\)
- Cattle, sheep and goats are the primary reservoirs for Q fever. Infection in humans most often occurs after inhalation of aerosolized organisms or with ingestion of raw milk or fresh goat cheese. \(^3,5\)

**Disease (Q fever)**

- Q fever in humans usually is asymptomatic or manifests as a mild disease with spontaneous recovery, and remains primarily as an occupational hazard in persons in contact with domestic animals. However, Q fever may lead to serious complications and even death in patients with acute disease, especially those with meningoencephalitis, myocarditis and endocarditis. \(^3\)
- *Coxiella burnetii* infection may lead to either acute or chronic Q fever, though almost 60% of Q fever cases are asymptomatic. The symptoms of acute Q fever include: fever, fatigue, chills, headache, myalgia, sweets, cough, nausea, vomiting, chest pain, diarrhea, skin rash, myocarditis, pericarditis, meningoencephalitis and death. The symptoms of chronic fever include: endocarditis, vascular infections, chronic hepatitis, pulmonary infections and fatigue. \(^3\)
- Stages: Incubation period for Q fever varies from 1 to 3 weeks depending on the number of organisms that initially infect the patient. Most patients become ill within 2-3 weeks after exposure. \(^1\)

The only report on bacteria burden over an extended period of time post infection is found in Meghari et al.\(^6\) Based on this study, we have divided the *C. burnetii* infection into 3 stages.
1. Incubation/development: 0-7 DPI (days post infection) (Stage 1)
2. Fulminant stage: 8-14 DPI (stage 2)
3. Clearing stage: 15 DPI and beyond (stage 3).

- Traditional treatments are antibiotics such as doxycycline, quinolone, streptomycin and hydroxy chloroquine.¹
- Prevention of Q fever: Since persons in contact with animals, such as farmers, veterinarians, meat processing plant workers are primarily at risk, prevention should be directly to these groups.
  A vaccine has been developed, but is not available in the US. ⁶

**BACTERIAL DISTRIBUTION**

While there are numerous animal studies on the pathology or pathogenesis of Q fever, many of them only report qualitative results on the distribution of *C. burnetii* in organs/tissues.⁷⁻¹⁰ Some report quantitative measurement of antibody titers against the *C. burnetii*, rather than bacterial loads directly.⁷,¹¹,¹² Summarized in the following are the quantitative bacterial distribution data based on a few papers that have included direct quantitative bacterial load information.

**Mouse**

**Study 1**: To compare the pathogenicities of *C. burnetii* in immunodeficient and immunocompetent mice, severe combined immunodeficient (SCID) mice and immunocompetent mice (C.B-17 and A/J mice) were inoculated intraperitoneally with 10 TCID50 of Nine Mile I strain of *C. burnetii*. It was found that the LD50 of *C. burnetii* in the SCID mice was at least 1E8 times less than that in the immunocompetent mice. Bacteria loading was reported qualitatively, however, in the discussion section of the paper, it stated that the amount of *C. burnetii* in the spleen of SCID mice was ~1E7 TCID50/g. It was not clear from this paper at which stage of the disease the bacteria load was measured.¹³

**Study 2**: In this study, both SCID and BALB/c mice were inoculated with Nine Mile I strain of *C. burnetii* bacteria via an aerosol route. *C. burnetii* loading in different organs were measured microscopically. In Table 1, bacteria counts in lungs of BALB/c mice are shown.¹⁴ To convert bacteria/mm(2) to bacteria/mL tissue, it was found that the sections cut for microscopic image analysis was 5 micron thick.¹⁵ As a 5 micron thick section of 1mm(2) is 5*10e(-6) mL in volume, dividing the numbers from the above table by this number (5*10e(-6)) (bacteria/mm(2)) gives the following result in bacteria/mL:

BALB/c mice: on day 7, bacteria in lung is 3.15/[5*10e(-6)] = 6E5; on day 14, it is 5E4. For SCID mice: on day 7, bacteria in lung is 1E6; on day 14, it is 4E5. Data on BALB/c mice was tabulated in Table 1.
Table 1. Bacterium counts in lungs of BALB/c mice infected with 10^8 Nine Mile strain organisms.

<table>
<thead>
<tr>
<th>days post infection</th>
<th>bacteria/mm(2) +/- SD</th>
<th>bacteria/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>3.15+/-1.11</td>
<td>6.E+05</td>
</tr>
<tr>
<td>14</td>
<td>0.24+/-0.12</td>
<td>5.E+04</td>
</tr>
</tbody>
</table>

Data used for the BioDMET database: mouse lung (stage 1): 6E5 ; mouse lung (stage 2): 5E4. Only data on BALB/c mice were reported here. SCID data not included.

Study 3: In this study, Nine Mile C. burnetii phase II infection was studied in BALB/c, C57BL/6 and immunodeficient mice to see if C. burnetii phase II cause disease in immunodeficient mice. Bacteria load was measured using quantitative PCR to determine genomic DNA copy numbers in organs.  

Note: C. burnetii phase II is the avirulent form, though bacteria burden in the spleen is fairly high.

C. burnetii NMII in mice was detected by quantitative PCR at 10 days post infection. The data was presented in Figure 4 of the paper. For the purpose of our study, only data on immunocompetent mice were of interest. From this Figure, the bacteria load in the spleens of BALB/c mice was 1E8/spleen, and that of C57BL/b mice was 5E5/spleen.

Data entered into Symptoms: mouse spleen (stage 2): 5E7 organism/g. Data entered are based on immunocompetent mice: BALB/c and C57BL/6.

Study 4: To understand the roles of immune components in animals infected with Nine Mile phase I (NMI) or phase II (NMII) bacteria, immunodeficient mice were compared with immunocompetent mice for their response to infection in this study. Bacteria load was measured using quantitative PCR to measure genome copies in spleen.

Bacterial genome numbers (B) in the spleen of mice infected with the low and high doses of NMI or NMII were measured at 28 DPI, and data were presented in Figures 2, 4 & 7 of this paper. Data on wild type mice (CB17) were summarized here in Table 2.

Table 2. Bacteria load in spleens of wild type mice after challenges with NMI and NMII Coxiella burnetii.

<table>
<thead>
<tr>
<th>Pathogen and dose</th>
<th>genome copy/spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMI, 1E2 genome/mouse</td>
<td>1.E+07</td>
</tr>
<tr>
<td>NMI, 1E5 genome/mouse</td>
<td>5.E+06</td>
</tr>
<tr>
<td>NMII, 1E2 genome/mouse</td>
<td>0.E+00</td>
</tr>
<tr>
<td>NMII, 1E5 genome/mouse</td>
<td>0.E+00</td>
</tr>
</tbody>
</table>
Data entered into Symptoms: spleen: b-ms: 1E7/spleen (stage 3); b-ms: 5E6/spleen (stage 3). Only data on wild type mice (CB17) is reported here.

Study 5: The goal of this study is to investigate the influence of age of animals on *C. burnetii* infection. Bacteria burden in the spleens of 1 month old mice was compared with that of 14 month old mice.  

Young and mature mice were infected with *C. burnetii* for 7 days, and bacteria in the spleen were revealed by immunostaining as shown in Table 3. The original bacteria counts in bacteria/mm(2) (Figure1 in the paper) were converted to bacteria/mL, similar to Study 2 as shown above.

Table 3. Bacteria load in spleens of young and old mice 7 days after being challenged with *Coxiella burnetii*.

<table>
<thead>
<tr>
<th>Mice type</th>
<th>bacteria/mm(2)</th>
<th>bacteria/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>1</td>
<td>2.E+05</td>
</tr>
<tr>
<td>Mature</td>
<td>5</td>
<td>1.E+06</td>
</tr>
</tbody>
</table>

Study 6: Mice over expressing interleukin IL-10 showed increased susceptibility to *C. burnetii* infection as compared to wild-type mice. In this study, both transgenic and wild-type mice were infected with *C. burnetii* by intraperitoneal and intratracheal routes and bacteria load in organs were quantified using real-time PCR over 42 days post infection. The results are shown in Figure 1 (intraperitoneal) & 6(intratracheal) of the original paper.

Summarized in Table 3 are the bacterial loads in different organs of wild-type mouse.

Table 3. Bacteria load in organs of wild type mouse after being challenged with *Coxiella burnetii* intraperitoneally and intratracheally.

### intraperitoneal DNA copies

<table>
<thead>
<tr>
<th>DNA copies</th>
<th>day 4</th>
<th>day 7</th>
<th>day 14</th>
<th>day 28</th>
<th>day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>spleen</td>
<td>5.E+04</td>
<td>3.E+05</td>
<td>1.E+05</td>
<td>2.E+04</td>
<td>1.E+04</td>
</tr>
<tr>
<td>liver</td>
<td>2.E+05</td>
<td>3.E+05</td>
<td>3.E+05</td>
<td>5.E+04</td>
<td>2.E+04</td>
</tr>
</tbody>
</table>

### intrachatracheal DNA copies

<table>
<thead>
<tr>
<th>DNA copies</th>
<th>day 1</th>
<th>day 7</th>
<th>day 14</th>
<th>day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>2.E+04</td>
<td>3.E+04</td>
<td>2.E+02</td>
<td>0.E+00</td>
</tr>
</tbody>
</table>
Data entered into Symptoms:
- Mouse spleen: 5E4/organ (stage 1); 3E5/organ (stage 2); 1E4/organ (stage 3).
- Mouse liver: 2E5/organ (stage 1); 3E5/organ (stage 2); 2E4/organ (stage 3).
- Mouse lung: 8E4/organ (stage 1); lung: 1E5 (stage 2); lung: 1E4 (stage 3). (intraperitoneal infection)

Mouse lung: 2E4/organ (stage 1); 2E2/organ (stage 2). (intratracheal infection)

Only data from wild type mice were enter into the Symptoms sheet.

Study 7: To elucidate the mechanisms of vaccine-induced protective immunity against *Coxiella burnetii* infection, the efficacy and immunogenicity between formalin-inactivated phase I vaccine (PI-V) and phase II vaccine (PII-V) in BALB/c were compared in mice. Mice were immunized using PI-V and PII-V, along with unvaccinated controls, and both groups were challenged with Nine Miles I (NMI) strain of *Coxiella Burnetii*. Bacteria loading in mice spleens was measured using real-time PCR and reported in Figure 3 of the paper. DNA copies/spleen from unvaccinated controls are listed here: on day 3: 1E4 copies of DNA/spleen; on day 7: 1E6 copies of DNA/spleen; on day 14, 1E6 copies of DNA/spleen. 18

Data entered into Symptoms: spleen: b-ms: 1E4/spleen (stage 1); spleen: b-ms: 1E6/spleen (stage 2).

References


