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SENTINEL LABORATORY GUIDELINES FOR SUSPECTED AGENTS OF BIOTERRORISM

Coxiella burnetii

American Society for Microbiology

Credits: *Coxiella burnetii*

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I. GENERAL INFORMATION

A. Description of organism

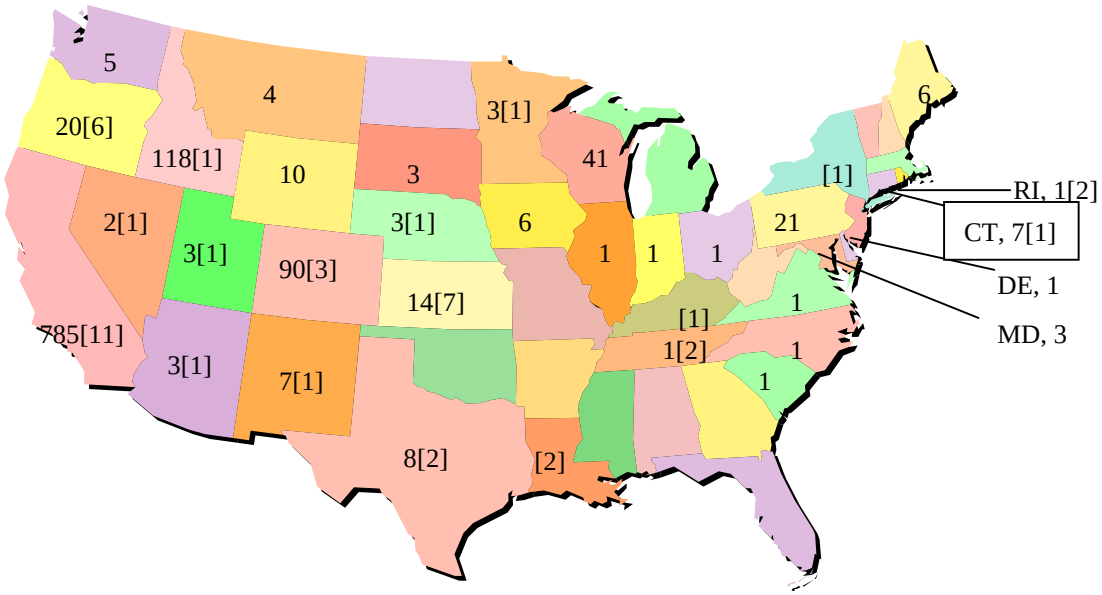
Coxiella burnetii is a pleomorphic coccobacillus that is gram-negative, obligately intracellular, and 0.3 to 0.7µm long. There is a sporelike form, the small cell variant, which is remarkably stable in extracellular environments. A large cell variant also exists that is the vegetative, metabolically active form. Mixtures of both forms are found in phagolysosomes. There is phase variation, similar to that in *Salmonella*, in which the lipopolysaccharide (LPS) varies chemically as either the virulent, phase I "smooth" type LPS, or the phase II "rough" LPS, associated with avirulent *C. burnetii*. *C. burnetii* is phylogenetically related to *Pseudomonas*, *Francisella*, and *Legionella*, within the *Legionella* group of the γ-*Proteobacteria* subdivision. It is more distantly related to *Rickettsia* (5).

B. History

A febrile illness among slaughterhouse workers was identified in Queensland, Australia, in 1935, and called "Query (Q)" fever because its etiology was unknown. The causative agent was originally named *Rickettsia diaporica* and then *Rickettsia burnetii* (7). Frank MacFarlane Burnet was a virologist who worked on the Australian cases. Also, in 1935, at the Rocky Mountain Laboratory in Hamilton, Montana, Herold R. Cox, a rickettsiologist, was among a group who investigated a tick-borne agent that resulted in a laboratory-acquired infection. It was designated the "Nine Mile agent," based on the source of the ticks being near Nine Mile creek. The Q fever agent and Nine-Mile agent were proven to be identical, and the agent was subsequently renamed *Coxiella burnetii* in honor of these two investigators (6, 9). Q fever is a zoonotic disease, especially of parturient goats, sheep, or cattle and occasionally domestic cats. Aerosolized contaminated dust particles are the source of human infection, and the infectious dose is very low. Infection may be acquired less commonly through ingestion of contaminated milk. Although ticks can be infected with *C. burnetii*, they do not represent a major vector of human disease, and human-to-human transmission is rare. On a worldwide basis, *C. burnetii* is the leading cause of overt laboratory-acquired infections among viral, rickettsial, and chlamydial agents. Most of these have involved work with pregnant sheep in research laboratories (4).

C. Geographic distribution

FIG. 1. Reported human Q fever cases, 1948-1977 and 2000



Active surveillance of Q fever has been inconsistent; however, Figure 1 shows the number of cases reported to the Centers for Disease Control and Prevention (CDC) by State Health Departments from 1948 to 1977, followed in brackets by the number of cases identified in 2000 by the CDC and physician network sources (2, 8). This serves to illustrate the distribution that has been experienced historically with cases of Q fever, as well as the more recent distribution of the disease. In conjunction with the emerging infections network of the Infectious Diseases Society of America, the CDC accounted for 45 cases of Q fever in the United States during 2000 (Q fever again became reportable in 1999), but this is still believed to have underrepresented the prevalence of disease. Where there has been active surveillance for the disease, it has been found.

D. Clinical presentation

The symptoms of Q fever are generally nonspecific. There are multiple presentations, most commonly pneumonia (47 to 63%), hepatitis (60%), or fever only (14%) (9). It is estimated that self-limited febrile illness may, in fact, be the most common form of the disease. The incubation period is 2 to 3 weeks. The organisms proliferate in the lung following inhalation of contaminated aerosols and then invade the bloodstream. Acute Q fever is characterized by sudden onset of high fever, headache, myalgias, arthralgias, cough, and, less frequently, rash or a meningeal syndrome. In addition to radiographic manifestations of pneumonia, patients often have elevated liver enzyme levels and erythrocyte sedimentation rates and thrombocytopenia. Development of chronic Q fever is a more serious disease, which can occur up to 20 years after the initial infection. The major complication of chronic Q fever is endocarditis. Overall, the mortality rate of Q fever is low, approximately 2.4% (10), but it may be as high as 65% among those with chronic Q fever (9).

Several potential bioterrorism agents could present as community-acquired pneumonias, including those causing tularemia, plague, anthrax, or Q fever. The milder forms of pneumonic tularemia could be clinically indistinguishable from Q fever, whereas plague or anthrax would typically follow a more rapidly fulminate course. In addition to atypical pneumonia as part of a clinical algorithm leading to consideration of Q fever the following should suggest Q fever (3): influenza-like illness during periods of low influenza activity; hepatitis without markers for hepatitis A, B, or C; and fever of unknown origin in children.

E. Treatment

Acute Q fever is most effectively treated when doxycycline is administered within 3 days after onset of the illness. Chronic Q fever endocarditis carries a poor prognosis and is much more difficult to treat. Combination long-term therapy with doxycycline and hydroxychloroquine or doxycycline with a fluoroquinolone is currently recommended (1).

II. PROCEDURES

A. General

Since this organism is an obligate intracellular organism, it cannot be cultured on routine bacteriologic media. The laboratory diagnosis of Q fever is based mainly on serologic testing. Antibody responses are measured against phase I and II antigens of *C. burnetii*. Patients with acute Q fever typically produce an antibody response primarily to *C. burnetii* phase II antigen, while chronic *C. burnetii* infections typically elicit a higher antibody response to phase I antigen. The diagnosis can be confirmed by (i) demonstration of fourfold or greater changes in antibody titer between paired acute- and convalescent-phase serum samples by immunofluorescence antibody testing, (ii) detection of *C. burnetii* by polymerase chain reaction or immunohistochemical staining of biopsy material from affected organs, or (iii) culture of this material (3). The demonstration of a single positive immunoglobulin G (IgG) or IgM titer (as defined by the testing laboratory) in clinically compatible cases defines a probable case of Q fever. A suggestive clue to the diagnosis of acute Q fever hepatitis is the presence of doughnut granulomas in liver (6), and a clue to the diagnosis of chronic Q fever is culture-negative endocarditis (3).

B. Precautions

Because of the highly infectious nature of this organism (Biosafety Level 3 [BSL-3]), specimens from suspected cases of Q fever should be immediately forwarded to a Local or State Health Department for isolation and identification. Due to the extreme infectivity of *C. burnetii*, Level A (Sentinel) laboratories should not attempt to culture this organism, but should be aware of the potential for inadvertent isolation of *C. burnetii* in cell culture systems designed for virus isolation (Fig. 2). *C. burnetii* can be inadvertently isolated in conventional cell cultures in a wide variety of cell lines, including all fibroblast cell lines. After an incubation period of 5 to 15 days, *C. burnetii*-infected cells are detectable as cytoplasmic inclusions (Fig. 2, arrows).

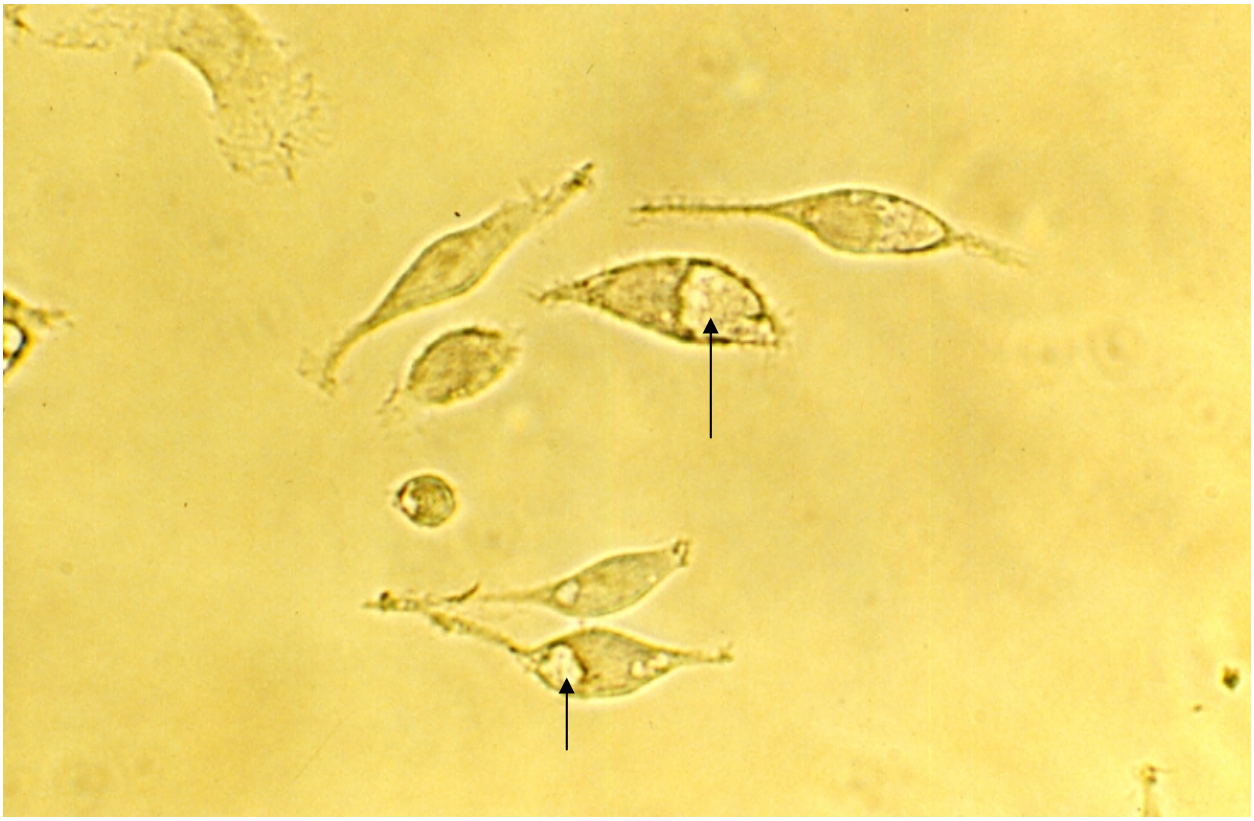


FIG. 2. Fibroblast L929 cell line infected with *Coxiella burnetii.*

Special decontamination procedures are necessary for surfaces potentially contaminated with *C. burnetii*. Household bleach solutions may be ineffective. Minor spills should be covered with absorbent paper, such as paper towels, and then flooded with 70% ethanol or 5% MicroChem-Plus (a dual quaternary ammonium compound), which should be allowed to act for 30 min before cleanup. Spills that involve samples with high concentrations of organisms, involve organic matter, or occur in areas of lower temperatures (e.g., refrigerators or freezers), should be exposed to disinfectant solution for 1 h before cleanup.

C. Specimens

1. Acceptable specimens. NOTE: Level A (Sentinel) laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the State Health Laboratory.

a. Serum. Collect serum (red-top or serum separator tube [SST], tiger-top tube) as soon as possible after onset of symptoms (acute phase) and with a follow-up specimen (convalescent phase) at $\geq$14 days for serological testing.

b. Blood. Collect blood in EDTA (lavender) or sodium citrate (blue) and maintain at 4^{o}C for storage and shipping for PCR or special cultures. If possible, collect specimens prior to antimicrobial therapy.

c. Tissue, body fluids, and others, including cell cultures and cell supernatants. Specimens can be kept at 2 to 8^{o}C if transported within 24 h. Store frozen at –70^{o}C or on dry ice.

2. Specimen handling

a. In conjunction with instructions from the State Public Health Laboratory, arrange for immediate shipment at 2 to 8°C to an appropriate higher-level Laboratory Response Network (LRN) laboratory.

b. Follow infectious substance regulations for packing and shipping.

c. Level A (Sentinel) laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the State Health Laboratory.

3. Rejection criteria

a. Incomplete documentation. All specimens must include the sender's name and telephone number to contact for the preliminary report and additional information.

b. Improper packaging/shipping

c. Lack of prior approval. Do not ship specimens to Level B or C (LRN Reference) laboratories without prior approval.

D. Reporting

1. Level A (Sentinel) laboratories should consult with the State Public Health Laboratory Director (or designate) prior to or concurrent with testing if *C. burnetii* is suspected by the attending physician.

2. Serology is available through commercial reference as well as public health laboratories. Report positive results to the patient's physician and hospital infection control and public health officials.

ACKNOWLEDGMENT

Didier Raoult kindly provided Fig. 2.

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