Chapter 10

Q FEVER

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INTRODUCTION

Q fever was discovered in Australia and in the United States before the outbreak of World War II. In Australia the disease was common in slaughterhouse workers and farm workers,[1] and it persists as an occupational problem.[2] This zoonotic disease is nearly worldwide and the etiologic agent, *Coxiella burnetii*, has a broad host range. Acute Q fever, although rarely life-threatening, can be temporarily incapacitating. Humans usually contract the disease by inhaling barnyard dust contaminated after parturition by infected animals. A single microorganism is sufficient to cause infection. The infectious particle is extremely resistant to environmental degradation. Acute disease is not accompanied by unique symptoms. Therefore, Q fever must be considered in the differential diagnosis when a history of animal contact is established. Rarely, acute Q fever progresses to chronic Q fever, a debilitating, life-threatening infection that is difficult to treat. Because of its high infectivity and stability in the environment, *C burnetii* is listed as a Category B biothreat agent.

HISTORY

In 1933 a disease of unknown origin was first observed in slaughterhouse workers in Queensland, Australia. Patients presented with fever, headache, and malaise. Serologic tests for a wide variety of possible etiologic agents were negative.[1] Because the disease had an unknown etiology, it was given the name Q fever (for query). The infection was shown to be transmissible when blood and urine from patients elicited a febrile response after injection into guinea pigs. The infection could be passed to successive animals. Unfortunately, no isolate could be obtained after culture on bacteriological media, and the etiologic agent was thought to be a virus.

About this time, ticks were being collected in western Montana as part of an ongoing investigation into Rocky Mountain spotted fever. Ticks collected from the Nine Mile Creek area caused a febrile response when placed onto guinea pigs. The infection could be passed to successive guinea pigs through injection of blood.[3] Examination of inflammatory cells from infected guinea pigs revealed rickettsia-like microorganisms, although the disease in guinea pigs was not spotted fever.[4] A breakthrough in cultivating this agent occurred with the discovery that it would grow in yolk sacs of fertilized hens' eggs.[5] Although the microorganism was demonstrated to be infectious, the disease it caused was unknown. In Australia, however, a disease was identified, but it had an unknown etiology.

In Montana a researcher was infected while working with the Nine Mile isolate, and guinea pigs could be infected by injecting a sample of the patient's blood. At the same time, infected mouse spleens were sent from Australia to the United States. In a remarkable mix of serendipity and science, it was confirmed that the agent causing Q fever and the Nine Mile isolate were the same by demonstrating that guinea pigs previously challenged with the Nine Mile isolate were resistant to challenge with the Q fever agent.[6] The conclusion could also be made that ticks transmitted Q fever. Although initially named *Rickettsia diaporica*[7] and *Rickettsia burnetii*,[8] the microorganism was given the name *C burnetii* in 1948 in honor of Dr Cox and Dr Burnet, who made important contributions regarding propagation and isolation of this agent.[9]

Investigations of Q fever soon established that *C burnetii* was prevalent in slaughterhouses and hazardous in the laboratory, and also could be spread by aerosol.[10,11] The successful culture of the Q fever organism in chicken embryos proved to be a fortuitous breakthrough for advances in Q fever research, as well as for other rickettsial organisms.[12] Q fever has been identified in over 50 countries.[13]

MILITARY RELEVANCE

An atypical pneumonia, similar to Q fever, was noted in German soldiers in Serbia and southern Yugoslavia during World War II.[14] The agent causing "balkengrippe" was not confirmed by laboratory testing, but the clinical and epidemiological features of the illness described were most consistent with Q fever. Hundreds of cases were observed in German troops in Italy, Crimea, Greece, Ukraine, and Corsica. Five Q fever outbreaks were also noted in American troops in Europe during the winter of 1944 and the spring of 1945.[14] Cases usually occurred in troops occupying farm buildings recently or concurrently inhabited by farm animals.[15] However, cases also occurred in the absence of close contact with livestock. At an airbase in southern Italy, 1,700 troops became infected, presumably as a result of infected sheep and goats being pastured nearby.[16]

More recent Q fever cases in military service members have also occurred. An acute Q fever outbreak associated with a spontaneous abortion epidemic in sheep and goats occurred in British troops deployed in Cyprus, American airmen in Libya, and French

soldiers in Algeria, causing 78 cases of illness.[14,17,18] Q fever outbreaks were also reported in Swiss and Greek soldiers and Royal Air Force airmen.[14] Q fever has been identified in American military personnel in the Persian Gulf War. One case of meningoencephalitis associated with acute Q fever was reported in a soldier who recently returned from the Persian Gulf.[19] Subsequent serologic testing in the author's laboratory identified three additional acute seroconversions in soldiers of the same battalion. These reports underscore the necessity of considering the possibility of Q fever in service members having symptoms consistent with a Q fever and a recent history of exposure to livestock that may harbor *C burnetii.*

INFECTIOUS AGENT

C burnetii is an obligate intracellular pathogen of eukaryotic cells and replicates only within the phagolysosomal vacuoles of host cells, primarily macrophages. Growth does not occur on any axenic medium. During natural infections, the organism grows to high titer in placental tissues of goats, sheep, and possibly cows.[20,21] This microorganism is routinely cultured in chicken embryo yolk sacs and in cell cultures,[22] and it can also be recovered in large numbers within spleens of experimentally infected mice and guinea pigs.[22] Growth is slow, with a generation time longer than 8 hours.[23]

The microorganism usually grows as a small coccobacillus, approximately 0.8 to 1.0 μm long by 0.3 to 0.5 μm wide. Like other gram-negative microorganisms, *C burnetii* possesses a lipopolysaccharide (LPS), although the Gram stain reaction is variable.[24,25] LPS is important in virulence and is responsible for the antigenic phase variation seen in this organism.[26,27] *C burnetii* can display LPS variations similar to the smooth-rough LPS variation in *Escherichia coli.*[26] Bacterial isolates from eukaryotic hosts have a phase I (smooth) LPS character, which can protect the organism from microbicidal activities of the host. As those isolates are passed in yolk sacs or other nonimmunocompetent hosts, the phase I LPS character of the bacterial population gradually changes to the phase II (rough) form. Phase I microorganisms are virulent, and phase II microorganisms are avirulent in immune competent hosts.

The developmental cycle features small, compacted cell types within mature populations growing in animal hosts.[28] These forms, called small cell variants (SCVs), are responsible for the organism's high infectivity, as well as its capability to survive relatively extreme environmental conditions; its chemical resistance; and its resistance to desiccation, heat, sonication, and pressure.[29] The large cell variants (LCVs) are probably the metabolically active cells of this organism. The SCV and LCV are antigenically different.[30] Transition between SCV and LCV does not involve classical phase variation, which refers to LPS structure, but can be accompanied by changes in the expression of surface protein.

Coxiella is an obligate intraphagolysosomal parasite with acid-activated metabolism, presumably because most of its transport mechanisms required for import of required nutrient substrates from the vacuole environment function in a pH range of 4.0 to 5.5. Purified organisms incubated without any host fractions or cells require an acid pH to transport or metabolize either glucose or glutamate.[31] However, in-vitro growth under acidic conditions has not resulted in axenic growth, although protein synthesis can occur. Growth in the harsh phagolysosomal environment shows that this microorganism has coping strategies. The coping mechanism, although undefined, may involve the production of oxygen scavengers.[32] An iron/manganese superoxide dismutase has been demonstrated, and genetic sequencing has also revealed a copper-zinc dismutase.[33] Because *C burnetii* is susceptible to reactive oxygen and nitrogen intermediates produced in response to infection by the host cells,[34] the microorganism's primary strategy for surviving within host cells is likely avoiding host cell activation. That phase I *C burnetii* does not activate human dendritic cells,[35] and that *C burnetii* LPS does not activate host antimicrobial responses via Toll-like receptor 4, are evidence to support this strategy.[36]

Disinfection

Ten percent household bleach did not kill the organisms during a 30-minute exposure.[37] Likewise, exposure to 5% Lysol, 2% Roccal, or 5% formalin for 30 minutes did not inactivate *C burnetii.*[37] The organism was inactivated within 30 minutes by exposure to 70% ethyl alcohol, 5% chloroform, or 5% Enviro-Chem.[37] (The latter chemical, a formulation of two quaternary ammonium compounds, is known as Micro-Chem Plus and is available through National Chemical Laboratories, Philadelphia, Pa.) Formaldehyde gas can also be an effective sterilizing agent when administered in a humidified (80% relative humidity) environment.[37]

Pasteurization

The frequent presence of *C burnetii* in cow's milk led to the establishment of effective milk pasteurization procedures. Temperatures of 61.7ºC for 20 minutes can kill the organisms in raw milk.[38] In the laboratory, aqueous suspensions of the microorganism are typically killed by treating at 80ºC for 1 hour.

Irradiation

Gamma irradiation can be used to sterilize biological preparations. The amount of gamma irradiation that reduced infectivity by 90% was 8.9×10^4 rads for *C burnetii* suspended in yolk sacs and 6.4×10^4 rads for the purified specimen.[39] The sterilizing dose was calculated to be 6.6×10^5 rads. Typically an irradiation dose of 2.1×10^6 rads is used for sterilizing serum samples. An important consideration is that useful biological specimens are not degraded after activation by irradiation. Gamma irradiation (2.1×10^6 rads) was shown to have no deleterious effect on the antibody-binding capacity of *C burnetii* antigen, the antigen-binding capability of anti-*C burnetii* antibody, the morphological appearance of *C burnetii* by electron microscopy, or the distribution of a major surface antigen.[39]

DISEASE

Epidemiology

Q fever is a zoonotic disease that occurs worldwide. Of the variety of species that can be infected by *C burnetii*, humans are the only species to develop symptomatic disease. Human infections are primarily found in persons occupationally exposed, such as ranchers, veterinarians, and workers in meatpacking plants. Domestic ungulates, such as cattle, sheep, and goats, usually acquire and transmit *C burnetii*, and domestic pets (primarily cats) can be a primary source of human infection in urban environments.[40-42] Heavy concentrations of microorganisms are secreted in milk, urine, feces, and especially in parturient products of infected pregnant animals.[43] Because of the stability of this agent, dried, infectious particles in barnyards, pastures, and stalls can be a source of infection months later.[43] Infection is most commonly acquired by breathing infectious aerosols or contaminated dust.[44] Patients can also be infected by ingesting contaminated milk[45] and through the bite of an infected tick.[3] Infection can also occur in individuals not having direct contact with infected animals, such as persons living along a road used by farm vehicles[46] or those handling contaminated clothing.[47,48]

C burnetii is extremely infectious for humans. The infectious dose is estimated to be 10 microorganisms or fewer.[49] The route of infection may determine the clinical manifestations of the disease.[50] In most cases of infections acquired by ingesting the microorganism, acute Q fever is found primarily as a granulomatous hepatitis.[51] However, in patients infected by the aerosol route, Q fever pneumonia is more common.[52] The infectious doses have been shown to vary inversely with the length of the incubation period.[53] Person-to-person transmission has been reported, but is rare.[54] The rates of Q fever seropositivity vary. In Nova Scotia, where extensive seroepidemiological work has been done, 14% of tested human samples were positive.[55] Overall, the incidence of Q fever is underreported. For example, in Michigan, although the first two Q fever cases were not reported until 1984, a survey showed that 15% of the general population surveyed and 32% of goat owners had serologic evidence of infection.[56] The incidence of reported Q fever is higher now than in the 1990s, partly because of improved surveillance and more accessible testing.

Researchers find it controversial whether bacterial strains causing chronic Q fever are fundamentally different from strains causing acute Q fever. Some evidence suggested a link between genetic structure and the disease type (chronic or acute),[57] but other researchers thought that host-specific factors were more important.[58] The lack of a good chronic Q fever animal model made it difficult to resolve the question. However, a recent genetic analysis showed that groupings based on allelic differences of 159 *C burnetii* isolates from chronic Q fever cases were never found associated with acute disease.[59] This observation strengthens the case that the disease course in humans can be related to the strain of the infecting microorganism.

Pathogenesis

Q fever is an acute, self-limited systemic illness that can develop into a chronic, debilitating disease. Pathogenesis of infection in human disease is not well defined. Studies with animal models show that after initial infection of the target organ, the microorganism is engulfed by resident macrophages and transported systemically. The acidic conditions within the phagolysosome allow cell growth. Eventually proliferation within the phagolysosome leads to rupture of the host cell and infection of a new population of host cells. In animal models, the spleen and liver and other tissues of the reticuloendothelial system appear to be most heavily infected, which is likely the case in human infection. Chronic Q fever cases can arise years after the initial presentation. Animals frequently remain infected over their lifespans, with outgrowth of the microorganism occurring during conditions of immunosuppression, such as parturition,[60] or in laboratory animals that have been immunosuppressed.[61] One of the unresolved mysteries of Q fever is where the microorganism is "hiding out"

in the intervening time between recovery from human acute disease and the development of chronic disease. Another unresolved question is whether humans ever completely clear the microorganism after infection. *Coxiella* DNA has been found in the bone marrow of the majority of patients who had primary Q fever 12 years previously.[62] Asymptomatic animals may also harbor the microorganism.[63]

Infection (Coxiellosis) in Animals

Coxiellosis is a zoonosis that affects native and domestic animals. Animals are infected by biting ectoparasites, primarily ticks, and by inhaling infectious particles.[64] Nursing calves can also be infected via their mother's milk—over 90% of dairy herds in the northeastern United States were found to be infected with *C burnetii*, based on surveillance of bulk milk samples.[65] Pasteurization of milk products decreases the risk of human infection. Infected animals generally appear to be asymptomatic, except for a rise in the rate of spontaneous abortions.[66] Domestic ruminants are the primary source of infection for humans. Eradication of *Coxiella* infection in animal populations is difficult because infection rarely causes symptoms. Unlike in humans, infection in animals does not cause pathological changes in the lungs, heart, or liver. The site most often affected is the female reproductive system, primarily the placenta, where damage is minimal. However, infection results in shedding vast quantities of organisms into the environment, which becomes a source of infection for other animals and humans.

Sheep have been a source of infection at medical research institutions, where animals used in neonatal research have caused Q fever in humans.[67-69] However, unlike cattle and goats that tend to remain chronically infected,[70] sheep likely do not shed the organisms into the environment over a long period.[64,71,72] Therefore, *Coxiella* infection in sheep might be a transient infection with a spontaneous cure, similar to most Q fever cases in humans.[64] Abortion is seen more often in infected sheep and goats than in cows.[73]

Clinical Disease in Humans

The majority of human *C burnetii* infections are asymptomatic, especially among high-risk groups, such as veterinary and slaughterhouse workers, other livestock handlers, and laboratory workers.[74] The vast majority of the overt disease cases are acute Q fever. Fatalities in acute Q fever cases are rare, with fewer than 1% of cases resulting in death.[1] The incubation period can last a few days to several weeks, and the severity of infection varies in direct proportion to the infectious

dose.[53,75] There are no characteristic symptoms of Q fever, but certain signs and symptoms tend to be more prevalent. Fever, severe headache, and chills are the symptoms most commonly seen. Fever usually peaks at 40°C and lasts approximately 13 days.[76] Fatigue and sweats are also frequently found.[77] Cough, nausea, vomiting, myalgia, arthralgia, chest pain, hepatitis, and occasionally, splenomegaly, osteomyelitis, and meningoencephalitis are also associated with acute Q fever.[19,77] Blood tests show a normal white blood cell count, although thrombocytopenia or mild anemia may be present.[78] The erythrocyte sedimentation rate is frequently elevated.[79] Neurological symptoms, such as hallucinations, dysphasia, hemi-facial pain, diplopia, and dysarthria, have been described in an outbreak of acute Q fever.[78] The duration of symptoms increases with age.[76]

Pneumonia is a common clinical presentation of acute Q fever.[80] Atypical pneumonia is most frequent, and asymptomatic patients can also exhibit radiologic changes that are usually nonspecific and can include rounded opacities and hilar adenopathy.[40,81] Infection can also cause acute granulomatous hepatitis with corresponding elevations of the aspirate transaminase and/or alanine transaminase.[77] Elevations in levels of alkaline phosphatase and total bilirubin are seen less commonly.

Chronic Q fever is rarer, but also results in more deaths than acute Q fever. Patients with prior coronary disease or patients immunocompromised because of disease, such as AIDS, or therapy, such as immunosuppressive cancer therapy or antirejection therapy after organ transplant, are more at risk for developing chronic Q fever.[82,83] Endocarditis, primarily of the aortic and mitral valves,[84] is the most common manifestation of chronic Q fever; although chronic hepatitis[85] and infection of surgical lesions[86] have been seen. Approximately 90% of Q fever endocarditis patients have preexisting valvular heart disease.[87] Of those acute Q fever patients with cardiac valve abnormalities, as many as one third develop endocarditis.[88] Patients with chronic Q fever lack T-cell responses, resulting in an immune response inadequate to eradicate the microorganism. This immunosuppression of host cellular immune responses is caused by a cell-associated immunosuppressive complex.[89] This complex may cause immunosuppression by stimulating the production of prostaglandin E2 and high levels of tumor necrosis factor, which may also have deleterious effects on the host.[90-92] Patients with chronic Q fever also have an increase in interleukin 10 secretion.[93] Suppression of host immunity may allow persistence of the microorganism in host cells during the development of chronic Q fever. Other pathological effects of chronic Q fever include the presence of circulating immune complexes, resulting in glomerulonephritis.[94]

DIAGNOSIS

Serology

Q fever is difficult to distinguish because it lacks characteristic features. Diagnosis is usually based on clinical symptoms, a history of exposure to animals, and serologic testing. Although specific cellular immune responses may be suppressed in acute Q fever cases, humoral immune responses appear to continue unabated during infection.[95] Therefore, clinicians frequently encounter situations where a presumptive diagnosis of acute Q fever, based on nonspecific signs and serology, warrants a diagnosis of acute Q fever leading to therapeutic intervention.

The two antigenic forms of *C burnetii* that are important for serologic diagnosis of Q fever are the phase I (ie, virulent microorganism with smooth LPS [S-LPS]) and phase II (ie, avirulent microorganism with rough LPS [R-LPS]) whole-cell antigens.[96,97] Determining antibodies against phase I and phase II *C burnetii* can help distinguish acute and chronic Q fever.[95] Infection of humans produces characteristic serologic profiles by various antibody tests. Although the complement fixation assay is generally regarded as the most specific serologic assay for Q fever, the indirect fluorescent antibody assay, the microagglutination assay, and the enzyme immunoassay can provide positive results earlier in the course of an infection.[98] Most diagnostic laboratories use either the indirect fluorescent antibody assay or enzyme immunoassay (Table 10-1). Both tests are sensitive and specific.[99] The indirect fluorescent antibody assay is generally used when equipment or space is limited or when small numbers of samples are tested. An advantage of the indirect fluorescent antibody assay is the ability to use phase I and phase II antigens unpurified from their yolk sac growth medium. The enzyme immunoassay is highly sensitive, easy to perform, has great potential adaptability for automation, and can be applied in epidemiological surveys.[100] A disadvantage is the requirement for a more highly purified cellular antigen for enzyme im-

munoassay. Such purified antigens are not usually commercially available.

Patients with acute Q fever may be distinguished from patients with chronic Q fever based on serologic results. In sera from acute Q fever patients, the magnitude of antiphase II titers exceeds those of antiphase I titers (Table 10-2).[95] However, in chronic Q fever patients, the antiphase I titers exceed those of anti-phase II titers, and patients with chronic Q fever endocarditis can have high levels of serum IgA.

Culture

Bacterial culture is not recommended for routine diagnosis of Q fever because of the difficulties and hazards associated with this agent. However, in research settings, the isolation and characterization of new strains can result in significant contributions to the phylogenetic study of the genus. Two basic methods are used to isolate *C burnetii* from clinical specimens: propagation of the microorganisms (1) in cell culture monolayers[101] and (2) in rodents.[22] In the "shell vial" technique, a eukaryotic cell monolayer is infected with patient tissues free of contaminants, and the presence of *C burnetii* is detected by fluorescent antibody methods or polymerase chain reaction (PCR). Results obtained using this technique are subjective and should not be the basis for making clinical decisions, predicting patient prognosis, or determining the presence of microorganisms in environmental samples.

Isolation of *C burnetii* from clinical samples can also be accomplished by injection of tissue homogenates into immunocompetent animals, such as mice.[22] With this technique, crude estimates of bacterial number in the infected tissues can be made by diluting and injecting samples because only one infective microorganism is required for growth (resulting in seroconversion) in an animal host.[102] The high infectivity and low mortality caused by infection increase the chances

TABLE 10-1

ASSAYS FOR THE SERODIAGNOSIS OF Q FEVER

Serologic Tests	Advantages	Disadvantages
Indirect fluorescent antibody	Can use unpurified diagnostic antigens	Inconvenient to test large numbers of sera
Enzyme-linked immunosorbent assay	Can evaluate large numbers of sera; used in epidemiological surveys	Requires highly purified diagnostic antigens

TABLE 10-2

SEROLOGIC DIAGNOSIS OF Q FEVER

Magnitude of Serologic Titers	Diagnosis
Antiphase II titer > antiphase I titer	Acute Q fever
Antiphase II titer < antiphase I titer	Chronic Q fever

of a successful isolation. Furthermore, contaminants found associated with tissues generally do not pose a problem for successful isolation because the host immune response should facilitate clearance of those microorganisms. Animals injected with homogenized infected tissues are bled at weekly intervals, and spleen homogenates from antibody-positive mice are injected into a new set of mice to allow the microorganisms to propagate in the host in pure culture. After two to four animal passages, spleen cell suspensions are injected into embryonated eggs, and a *C burnetii* isolate is purified from the infected yolk sacs. Isolation of the Q fever etiologic agent is performed at research institutions engaged in studying the infectious agent and is unnecessary for diagnosing a case of Q fever in patients.

C burnetii can be identified in clinical samples, in infected cell cultures, or in infected lab animals by PCR.[103-105] The most useful PCR targets are those that use the insertion sequence IS1111.[106] Each *C burnetii* Nine Mile Creek strain chromosome contains at least 19 copies of this sequence, and every *C burnetii* isolate tested so far has multiple copies of this element. Human leukocytes obtained from citrated or EDTA blood can be used for determining the presence of *C burnetii*.[80] *C burnetii* DNA was identified in the sternal wound of a chronic Q fever endocarditis patient by PCR.[86]

TREATMENT

Although it is not bactericidal, doxycycline is the recommended treatment for human acute Q fever.[107] The recommended dose for treating acute disease in adults is 100 mg doxycycline, twice daily.[107] However, doxycycline or tetracyclines alone are not sufficient for treating chronic Q fever; drug combinations are needed, especially when endocarditis is present. One of the most efficacious treatments is doxycycline plus hydroxychloroquine.[108] Q fever endocarditis patients generally receive 18 months of therapy with doxycycline, 100 mg twice daily, and chloroquine, 200 mg three times daily.[107] Quinolones can also be used for those who cannot tolerate chloroquine. For these patients, 3 years of therapy with doxycycline, 100 mg twice daily, and ofloxacin, 200 mg three times daily, is recommended.[107] The long duration is recommended because relapses have occurred when the latter regimen was stopped.[108] Hydroxychloroquine probably enhances the efficacy of the doxycycline by making the phagolysosome alkaline, which restricts *Coxiella's* acidophilic metabolism.[109] Yeaman and Baca have reviewed unsuccessful results with single treatments of doxycycline and chloramphenicol for human endocarditis.[110] Recently, clarithromycin showed promise in acute Q fever clinical trials.[111] Strains of the microorganism that are resistant to antibiotics have been isolated.[112]

Evaluating antibiotic susceptibility of *C burnetii* isolates has been difficult because conventional methods cannot be used. An improved method has recently been developed using real-time PCR to determine bacterial replication in cells cultured in the presence and absence of antibiotics.[113]

PROPHYLAXIS

Control of *C burnetii* infection depends on stimulating a cell-mediated immune response, as is typical of microorganisms that grow intracellularly inside host cells.[114] Laboratory experiments have shown that stimulation of macrophage antimicrobial mechanisms by T-cell gamma interferon production leads to control of infection.[115,116] Passive transfer of antibodies did not control infection.[117] In addition, pretreating *C burnetii* with specific antibodies before infection also failed to control intracellular replication.[118]

An efficacious Q fever vaccine was developed and available for human vaccination only a few years after discovery of the etiologic agent. This preparation was rather crude, consisting of formalin-killed and ether-extracted *C burnetii* containing 10% yolk sack, but was effective in protecting human volunteers from disease after aerosol challenge.[119] The phase of the microorganism is important in efficacy of the vaccine. In the early studies, the antigenic nature of the vaccine was not known. More recent vaccines for Q fever are prepared from phase I microorganisms because those preparations are 100 to 300 times more potent than phase II vaccines.[120] Improved purification methods were eventually developed to exclude egg proteins and lipids. Vaccine efficacy of these more highly purified preparations was demonstrated in

human volunteers.[121] Although this and other early phase I cellular vaccines were efficacious, their use was occasionally accompanied by adverse reactions at the vaccination site, including induration or the formation of sterile abscesses or granulomas.[122] Previously infected or previously vaccinated individuals were at risk for developing these adverse reactions.[122] Approximately 3% of persons vaccinated for the ninth and tenth time developed severe persistent reactions.[123] The development and use of a skin test to exclude immune individuals from being vaccinated[124] resulted in a dramatic decrease in the incidence of adverse reactions after vaccination. Currently, skin testing is used to assess the potential for developing adverse vaccination reactions, although some laboratories also measure the level of specific antibodies against *C burnetii*.[125] Only individuals testing negative are vaccinated. Cellular *C burnetii* vaccines currently in use are safe and efficacious if the recipients are not immune before vaccination.

The most tested Q fever vaccine is Q-Vax (CSL Limited, Parkville, Victoria, Australia), a formalin-killed, phase I cellular vaccine that is produced and licensed for use in Australia.[125] In Australian studies, this vaccine has been 100% effective in preventing clinical Q fever in occupationally at-risk individuals, with the duration of protection exceeding 5 years.[125] However, the vaccine cannot be administered without prior determination of immunity. A similar product, which is not licensed, is administered as an Investigational New Drug. This vaccine is available through the US Army Medical Research Institute of Infectious Diseases for vaccinating at-risk persons in the United States.

Although attenuated microorganisms generally are not used as Q fever vaccines, a phase II attenuated strain, designated M-44, was developed from the Greek "Grita" strain in the former Soviet Union.[126] This vaccine can produce an adverse reaction and caused myocarditis, hepatitis, liver necrosis granuloma formation, and splenitis in guinea pigs.[127] Human vaccinees did not develop antiphase I antibodies, and antiphase II levels were variable and at low titer.

Potential difficulties may be encountered in evaluating immunity before vaccination. Using serologic titer as an indicator of immunity may not eliminate the risk of adverse vaccination reactions because specific antibody titers decrease after acute infection[128] and may not accurately reflect the immune status of the individual. Performing skin tests is time consuming and expensive, and the test might be incorrectly applied or misinterpreted. Therefore, efforts are underway to develop safer Q fever vaccines that will pose a lesser risk if given to someone with preexisting immunity. Such a vaccine could eliminate the requirement for prevaccination screening of potential vaccinees while retaining vaccine efficacy. With only a single visit to a healthcare practitioner needed, vaccination would be simpler and less expensive. One candidate vaccine was made by extracting phase I whole cells with a mixture of chloroform and methanol. The residue after extraction (chloroform-methanol residue vaccine; CMR) did not cause adverse reactions in mice at doses much higher than doses of phase I cellular vaccine that caused severe adverse reactions.[129] Efficacy of CMR vaccine has been demonstrated in laboratory rodents, sheep, and nonhuman primates.[130-133] Efficacious Q fever vaccines would benefit those occupationally at risk for Q fever, persons residing in areas endemic for Q fever, and soldiers or civilians who may be exposed due to a bioterrorist or biowarfare attack.

SUMMARY

Q fever is a zoonotic disease that is caused by the rickettsia-like organism *C burnetii*, which is important because of its exceptional infectivity. The disease is mainly transmitted by inhalation of infected aerosols, and a single organism may cause infection in humans. The disease is distributed worldwide, and the primary reservoir for human infection is livestock animals, particularly goats, sheep, and cattle. Contact with parturient animals or products of conception poses especially high risk because the organism is present in high numbers in this setting. The organism is also resistant to pressure and dessication, and it may persist in a spore-like form in the environment for months.

Diagnosis is performed by serologic testing. Treatment of acute Q fever with tetracyclines is effective. Prevention is possible with a formalin-killed, whole-cell vaccine, but prior skin testing to exclude immune individuals is necessary to avoid the potential of severe local reactions. A Q fever vaccine is licensed in Australia, yet a similar product remains investigational in the United States.

REFERENCES

1. Derrick EH. Q fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Rev Infect Dis.* 1983;5:790–800.

2. Marmion BP. *Q Fever: Your Questions Answered.* Sydney, New South Wales, Australia: MediMedia Communications and CSL Limited; 1999: 32.

3. Davis GE, Cox HR. A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni,* reactions in animals, and filtration experiments. *Public Health Rep.* 1938;53:2259–2261.

4. Cox HR. A filter-passing infectious agent isolated from ticks. III. Description of organism and cultivation experiments. *Public Health Rep.* 1938;53:2270–2276.

5. Cox HR, Bell EJ. The cultivation of *Rickettsia diaporica* in tissue culture and in tissues of developing chick embryos. *Public Health Rep.* 1939;54:2171–2178.

6. Dyer RE. A filter-passing infectious agent isolated from ticks. IV. Human infection. *Public Health Rep.* 1938;53:2277–2283.

7. Cox HR. Studies of a filter-passing infectious agent isolated from ticks. V. Further attempts to cultivate in cell-free media. Suggested classification. *Public Health Rep.* 1939;54:1822–1827.

8. Derrick EH. *Rickettsia burnetii:* the cause of "Q" fever. *Med J Aust.* 1939;1:14.

9. Philip CB. Comments on the name of the Q fever organism. *Public Health Rep.* 1948;63:58.

10. Huebner RJ. Report of an outbreak of Q fever at the National Institutes of Health. II. Epidemiological features. *Am J Public Health.* 1947;37:431–440.

11. Topping NH, Shephard CC, Irons JV. Epidemiologic studies of an outbreak among stock handlers and slaughterhouse workers. *JAMA.* 1947;133:813–815.

12. Cox HR. Cultivation of rickettsiae of the Rocky Mountain spotted fever, typhus and Q fever groups in the embryonic tissues of developing chicks. *Science.* 1941;94:399–403.

13. Kaplan MM, Bertagna P. The geographical distribution of Q fever. *Bull World Health Organ.* 1955;13:829–860.

14. Spicer AJ. Military significance of Q fever: a review. *J R Soc Med.* 1978;71:762–767.

15. Robbins FC, Gauld RL, Warner FB. Q fever in the Mediterranean area: report of its occurrence in allied troops. II. Epidemiology. *Am J Hyg.* 1946;44:23–50.

16. The Commission of Acute Respiratory Diseases, F. B., North Carolina. Epidemics of Q fever among troops returning from Italy in the Spring of 1945. III. Etiological studies. *Am J Hyg.* 1946:88–102.

17. Spicer AJ, Crowther RW, Vella EE, Bengtsson E, Miles R, Pitzolis G. Q fever and animal abortion in Cyprus. *Trans R Soc Trop Med Hyg.* 1977;71:16–20.

18. Rombo L, Bengtsson E, Grandien M. Serum Q fever antibodies in Swedish UN soldiers in Cyprus—reflecting a domestic or foreign disease? *Scand J Infect Dis.* 1978;10:157–158.

19. Ferrante MA, Dolan MJ. Q fever meningoencephalitis in a soldier returning from the Persian Gulf War. *Clin Infect Dis.* 1993;16:489–496.

20. Luoto L, Huebner RJ. Q fever studies in Southern California. IX. Isolation of Q fever organisms from paturient placentas of naturally infected dairy cows. *Public Health Rep.* 1950;65:541–544.

21. Welsh HH, Lennette EH, Abinanti FR, Winn JF. Q fever in California. IV. Occurrence of *Coxiella burnetii* in the placenta of naturally infected sheep. *Public Health Rep.* 1951;66:1473–1477.

22. Waag D, Williams J, Peacock M, Raoult D. Methods for isolation, amplification, and purification of *Coxiella burnetii.* In: Williams J, Thompson HA, eds. *Q fever: The Biology of Coxiella burnetii.* Boca Raton, Fla: CRC Press; 1991: 73–115.

23. Thompson HA. Relationship of the physiology and composition of *Coxiella burnetii* to the Coxiella-host cell interaction. In: Walker DH, ed. *Biology of Rickettsial Diseases.* Boca Raton, Fla: CRC Press; 1988: 51–78.

24. Schramek S, Mayer H. Different sugar compositions of lipopolysaccharides isolated from phase I and pure phase II cells of *Coxiella burnetii. Infect Immun.* 1982;38:53–57.

25. Amano K, Williams JC. Chemical and immunological characterization of lipopolysaccharides from phase I and phase II *Coxiella burnetii. J Bacteriol.* 1984;160:994–1002.

26. Stoker MG, Fiset P. Phase variation of the Nine Mile and other strains of *Rickettsia burnetii. Can J Microbiol.* 1956;2:310–321.

27. Moos A, Hackstadt T. Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. *Infect Immun.* 1987;55:1144–1150.

28. McCaul TF, Williams JC. Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. *J Bacteriol.* 1981;147:1063–1076.

29. Samuel JE. Developmental cycle of *Coxiella burnetii*. In: Brun YV, Shimkets LJ, eds. *Procaryotic Development.* Washington, DC: ASM Press; 2000: 427–440.

30. McCaul TF, Banerjee-Bhatnagar N, Williams JC. Antigenic differences between *Coxiella burnetii* cells revealed by postembedding immunoelectron microscopy and immunoblotting. *Infect Immun.* 1991;59:3243–3253.

31. Hackstadt T, Williams JC. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii. Proc Natl Acad Sci U S A.* 1981;78:3240–3244.

32. Akporiaye ET, Baca OG. Superoxide anion production and superoxide dismutase and catalase activities in *Coxiella burnetii. J Bacteriol.* 1983;154:20–23.

33. Heinzen RA, Frazier ME, Mallavia LP. *Coxiella burnetii* superoxide dismutase gene: cloning, sequencing, and expression in *Escherichia coli. Infect Immun.* 1992;60:3814–3823.

34. Brennan RE, Russell K, Zhang G, Samuel JE. Both inducible nitric oxide synthase and NADPH oxidase contribute to the control of virulent phase I *Coxiella burnetii* infections. *Infect Immun.* 2004;72:6666–6675.

35. Shannon JG, Howe D, Heinzen RA. Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. *Proc Natl Acad Sci U S A.* 2005;102:8722–8727.

36. Zamboni DS, Campos MA, Torrecilhas AC, et al. Stimulation of Toll-like receptor 2 by *Coxiella burnetii* is required for macrophage production of pro-inflammatory cytokines and resistance to infection. *J Biol Chem.* 2004;279:54405–54415.

37. Scott GH, Williams JC. Susceptibility of *Coxiella burnetii* to chemical disinfectants. *Ann N Y Acad Sci.* 1990;590:291–296.

38. Enright JB, Sadler WW, Thomas RC. Thermal inactivation of *Coxiella burnetii* and its relation to pasteurization of milk. *Public Health Monogr.* 1957;54:1–30.

39. Scott GH, McCaul TF, Williams JC. Inactivation of *Coxiella burnetii* by gamma irradiation. *J Gen Microbiol.* 1989;135:3263–3270.

40. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC. Poker players' pneumonia: an urban outbreak of Q fever following exposure to a parturient cat. *N Engl J Med.* 1988;319:354–356.

41. Laughlin T, Waag D, Williams J, Marrie T. Q fever: from deer to dog to man. *Lancet.* 1991;337:676–677.

42. Baca OG, Paretsky D. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. *Microbiol Rev.* 1983;47:127–149.

43. Welsh HH, Lennette EH, Abinanti FR, Winn JF. Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. *Ann NY Acad Sci.* 1958;70:528–540.

44. DeLay PD, Lennette EH, Deome KB. Q fever in California; recovery of *Coxiella burnetii* from naturally infected airborne dust. *J Immunol*. 1950;65:211–220.

45. Huebner RJ, Jellison WL, Beck MD, Parker RR, Shepard CC. Q fever studies in Southern California. I. Recovery of *Rickettsia burnetii* from raw milk. *Public Health Rep.* 1948;63:214–222.

46. Salmon MM, Howells B, Glencross EJ, Evans AD, Palmer SR. Q fever in an urban area. *Lancet.* 1982;1:1002–1004.

47. Oliphant JW, Gordon DA, Meis A, et al. Q fever in laundry workers presumably transmitted from contaminated clothing. *Am J Hyg.* 1949;49:76–82.

48. Marrie TJ, Langille D, Papukna V, Yates L. Truckin' pneumonia: an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. *Epidemiol Infect.* 1989;102:119–127.

49. Tigertt WD, Benenson AS, Gochenour WS. Airborne Q fever. *Bacteriol Rev.* 1961;25:285–293.

50. Marrie TJ, Stein A, Janigan D, Raoult D. Route of infection determines the clinical manifestations of acute Q fever. *J Infect Dis.* 1996;173:484–487.

51. Fishbein DB, Raoult D. A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. *Am J Trop Med Hyg.* 1992;47:35–40.

52. Marrie TJ, Durant H, Williams JC, Mintz E, Waag DM. Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada. *J Infect Dis.* 1988;158:101–108.

53. Benenson AS, Tigertt WD. Studies on Q fever in man. *Trans Assoc Am Physicians.* 1956;69:98–104.

54. Mann JS, Douglas JG, Inglis JM, Leitch AG. Q fever: person to person transmission within a family. *Thorax*. 1986;41:974–975.

55. Marrie TJ. Epidemiology of Q fever. In: Marrie TJ, ed. *Q fever: The Disease.* Boca Raton, Fla: CRC Press; 1990: 49–70.

56. Sienko DG, Bartlett PC, McGee HB, Wentworth BB, Herndon JL, Hall WN. Q fever: a call to heighten our index of suspicion. *Arch Intern Med.* 1988;148:609–612.

57. Samuel JE, Frazier ME, Mallavia LP. Correlation of plasmid type and disease caused by *Coxiella burnetii*. *Infect Immun.* 1985;49:775–779.

58. Yu X, Raoult D. Serotyping *Coxiella burnetii* isolates from acute and chronic Q fever patients by using monoclonal antibodies. *FEMS Microbiol Lett.* 1994;117:15–19.

59. Glazunova O, Roux V, Freylikman O, et al. *Coxiella burnetii* genotyping. *Emerg Infect Dis.* 2005;11:1211–1217.

60. Sidwell RW, Gebhardt LP. Studies of latent Q fever infections. 3. Effects of parturition upon latently infected guinea pigs and white mice. *Am J Epidemiol.* 1966;84:132–137.

61. Sidwell RW, Thorpe BD, Gebhardt LP. Studies of latent Q fever infections. II. Effects of multiple cortisone injections. *Am J Hyg.* 1964;79:320–327.

62. Marmion BP, Storm PA, Ayres JG. Long-term persistence of *Coxiella burnetii* after acute primary Q fever. *QJM.* 2005;98:7–20.

63. Harris RJ, Storm PA, Lloyd A, Arens M, Marmion BP. Long-term persistence of *Coxiella burnetii* in the host after primary Q fever. *Epidemiol Infect.* 2000;124:543–549.

64. Lang GH. Coxiellosis (Q fever) in animals. In: Marrie T, ed. *Q fever: The Disease.* Boca Raton, Fla: CRC Press; 1990: 23–48.

65. Kim SG, Kim EH, Lafferty CJ, Dubovi E. *Coxiella burnetii* in bulk tank milk samples, United States. *Emerg Infect Dis.* 2005;11:619–621.

66. Hatchette T, Campbell N, Hudson R, Raoult D, Marrie TJ. Natural history of Q fever in goats. *Vector Borne Zoonotic Dis.* 2003;3:11–15.

67. Meiklejohn G, Reimer LG, Graves PS, Helmick C. Cryptic epidemic of Q fever in a medical school. *J Infect Dis.* 1981;144:107–113.

68. Simor AE, Brunton JL, Salit IE, Vellend H, Ford-Jones L, Spence LP. Q fever: hazard from sheep used in research. *Can Med Assoc J.* 1984;130:1013–1016.

69. Ruppanner R, Brooks D, Franti CE, Behymer DE, Morrish D, Spinelli J. Q fever hazards from sheep and goats used in research. *Arch Environ Health.* 1982;37:103–110.

70. D'Angelo LJ, Baker EF, Schlosser W. From the Centers for Disease control: Q fever in the United States 1948–1977. *J Infect Dis.* 1979;139:613–615.

71. Babudieri B. Q fever: a zoonosis. *Adv Vet Sci.* 1959;5:81–154.

72. Schaal EH. Udder colonization with *Coxiella burnetii* in cattle Q fever [in German]. *Dtsch Tierarztl Wochenschr.* 1982;89:411–414.

73. Palmer NC, Kierstead M, Key DW, Williams JC, Peacock MG, Vellend H. Placentitis and abortion in goats and sheep in Ontario caused by *Coxiella burnetii. Can Vet J.* 1983;24:60–61.

74. Rauch AM, Tanner M, Pacer RE, Barrett MJ, Brokopp CD, Schonberger LB. Sheep-associated outbreak of Q fever, Idaho. *Arch Intern Med.* 1987;147:341–344.

75. Sawyer LA, Fishbein DB, McDade JE. Q fever: current concepts. *Rev Infect Dis.* 1987;9:935–946.

76. Derrick EH. The course of infection with *Coxiella burnetii. Med J Aust.* 1973;1:1051–1057.

77. Marrie T. Acute Q fever. In: Marrie T, ed. *Q Fever, The Disease*. Boca Raton, Fla: CRC Press; 1990: 125–160.

78. Smith DL, Ayres JG, Blair I, et al. A large Q fever outbreak in the West Midlands: clinical aspects. *Respir Med.* 1993;87:509–516.

79. Marrie TJ. *Coxiella burnetii* (Q fever) pneumonia. *Clin Infect Dis.* 1995;21(Suppl 3):S253–S264.

80. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. *J Clin Microbiol.* 1998;36:1823–1834.

81. Smith DL, Wellings R, Walker C, Ayres JG, Burge PS. The chest x-ray in Q fever: a report on 69 cases from the 1989 West Midlands outbreak. *Br J Radiol.* 1991;64:1101–1108.

82. Heard SR, Ronalds CJ, Heath RB. *Coxiella burnetii* infection in immunocompromised patients. *J Infect.* 1985;11:15–18.

83. Raoult D, Levy PY, Dupont HT, et al. Q fever and HIV infection. *AIDS.* 1993;7:81–86.

84. Tobin MJ, Cahill N, Gearty G, et al. Q fever endocarditis. *Am J Med.* 1982;72:396–400.

85. Yebra M, Marazuela M, Albarran F, Moreno A. Chronic Q fever hepatitis. *Rev Infect Dis.* 1988;10:1229–1230.

86. Ghassemi M, Agger WA, Vanscoy RE, Howe GB. Chronic sternal wound infection and endocarditis with *Coxiella burnetii. Clin Infect Dis.* 1999;28:1249–1251.

87. Brouqui P, Dupont HT, Drancourt M, et al. Chronic Q fever: ninety-two cases from France, including 27 cases without endocarditis. *Arch Intern Med.* 1993;153:642–648.

88. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risk factors and prevention of Q fever endocarditis. *Clin Infect Dis.* 2001;33:312–316.

89. Waag DM, Williams JC. Immune modulation by *Coxiella burnetii*: characterization of a phase I immunosuppressive complex differentially expressed among strains. *Immunopharmacol Immunotoxicol.* 1988;10:231–260.

90. Koster FT, Williams JC, Goodwin JS. Cellular immunity in Q fever: modulation of responsiveness by a suppressor T cell-monocyte circuit. *J Immunol.* 1985;135:1067–1072.

91. Mege JL, Maurin M, Capo C, Raoult D. *Coxiella burnetii*: the 'query' fever bacterium. A model of immune subversion by a strictly intracellular microorganism. *FEMS Microbiol Rev.* 1997;19:209–217.

92. Waag DM, Kende M, Damrow TA, Wood OL, Williams JC. Injection of inactivated phase I *Coxiella burnetii* increases non-specific resistance to infection and stimulates lymphokine production in mice. *Ann N Y Acad Sci.* 1990;590:203–214.

93. Capo C, Zaffran Y, Zugun F, Houpikian P, Raoult D, Mege JL. Production of interleukin-10 and transforming growth factor beta by peripheral blood mononuclear cells in Q fever endocarditis. *Infect Immun.* 1996;64:4143–4147.

94. Perez-Fontan M, Huarte E, Tellez A, Rodriguez-Carmona A, Picazo ML, Martinez-Ara J. Glomerular nephropathy associated with chronic Q fever. *Am J Kidney Dis.* 1988;11:298–306.

95. Peacock MG, Philip RN, Williams JC, Faulkner RS. Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. *Infect Immun.* 1983;41:1089–1098.

96. Dupuis G, Peter O, Luthy R, Nicolet J, Peacock M, Burgdorfer W. Serological diagnosis of Q fever endocarditis. *Eur Heart J.* 1986;7:1062–1066.

97. Peter O, Dupuis G, Burgdorfer W, Peacock M. Evaluation of the complement fixation and indirect immunofluorescence tests in the early diagnosis of primary Q fever. *Eur J Clin Microbiol.* 1985;4:394–396.

98. Williams JC, Thomas LA, Peacock MG. Humoral immune response to Q fever: enzyme-linked immunosorbent assay antibody response to *Coxiella burnetii* in experimentally infected guinea pigs. *J Clin Microbiol.* 1986;24:935–939.

99. Uhaa IJ, Fishbein DB, Olson JG, Rives CC, Waag DM, Williams JC. Evaluation of specificity of indirect enzyme-linked immunosorbent assay for diagnosis of human Q fever. *J Clin Microbiol.* 1994;32:1560–1565.

100. Behymer DE, Ruppanner R, Brooks D, Williams JC, Franti CE. Enzyme immunoassay for surveillance of Q fever. *Am J Vet Res.* 1985;46:2413–2417.

101. Raoult D, Torres H, Drancourt M. Shell-vial assay: evaluation of a new technique for determining antibiotic susceptibility, tested in 13 isolates of *Coxiella burnetii*. *Antimicrob Agents Chemother.* 1991;35:2070–2077.

102. Williams JC. Infectivity, virulence, and pathogenesis of *Coxiella burnetii* for various hosts. In: Williams JC, Thompson HA, eds. *Q Fever: The Biology of Coxiella burnetii.* Boca Raton, Fla: CRC Press; 21–71.

103. Frazier ME, Mallavia LP, Samuel JE, Baca OG. DNA probes for the identification of *Coxiella burnetii* strains. *Ann N Y Acad Sci.* 1990;590:445–458.

104. Stein A, Raoult D. Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. *J Clin Microbiol.* 1992;30:2462–2466.

105. Willems H, Thiele D, Frolich-Ritter R, Krauss H. Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). *Zentralbl Veterinarmed B.* 1994;41:580–587.

106. Hoover TA, Culp DW, Vodkin MH, Williams JC, Thompson HA. Chromosomal DNA deletions explain phenotypic characteristics of two antigenic variants, phase II and RSA 514 (crazy), of the *Coxiella burnetii* nine mile strain. *Infect Immun.* 2002;70:6726–6733.

107. Maurin M, Raoult D. Q fever. *Clin Microbiol Rev.* 1999;12:518–553.

108. Raoult D, Houpikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. *Arch Intern Med.* 1999;159:167–173.

109. Maurin M, Benoliel AM, Bongrand P, Raoult D. Phagolysosomes of *Coxiella burnetii*-infected cell lines maintain an acidic pH during persistent infection. *Infect Immun.* 1992;60:5013–5016.

110. Yeaman MR, Baca OG. Antibiotic susceptibility of *Coxiella burnetii*. In: Marrie T, ed. *Q Fever: The Disease.* Boca Raton, Fla: CRC Press; 1990: 213–223.

111. Gikas A, Kofteridis DP, Manios A, Pediaditis J, Tselentis Y. Newer macrolides as empiric treatment for acute Q fever infection. *Antimicrob Agents Chemother.* 2001;45:3644–3646.

112. Spyridaki I, Psaroulaki A, Kokkinakis E, Gikas A, Tselentis Y. Mechanisms of resistance to fluoroquinolones in *Coxiella burnetii. J Antimicrob Chemother.* 2002;49:379–382.

113. Brennan RE, Samuel JE. Evaluation of *Coxiella burnetii* antibiotic susceptibilities by real-time PCR assay. *J Clin Microbiol.* 2003;41:1869–1874.

114. Kishimoto RA, Rozmiarek H, Larson EW. Experimental Q fever infection in congenitally athymic nude mice. *Infect Immun.* 1978;22:69–71.

115. Turco J, Thompson HA, Winkler HH. Interferon-gamma inhibits growth of *Coxiella burnetii* in mouse fibroblasts. *Infect Immun.* 1984;45:781–783.

116. Scott GH, Williams JC, Stephenson EH. Animal models in Q fever: pathological responses of inbred mice to phase I *Coxiella burnetii. J Gen Microbiol.* 1987;133:691–700.

117. Humphres RC, Hinrichs DJ. Role of antibody in *Coxiella burnetii* infection. *Infect Immun.* 1981;31:641–645.

118. Kishimoto RA, Walker JS. Interaction between *Coxiella burnetii* and guinea pig peritoneal macrophages. *Infect Immun.* 1976;14:416–421.

119. Smadel JE, Snyder MJ, Robins FC. Vaccination against Q fever. *Am J Hyg.* 1948;47:71–78.

120. Ormsbee RA, Bell EJ, Lackman DB, Tallent G. The influence of phase on the protective potency of Q fever vaccine. *J Immunol.* 1964;92:404–412.

121. Fiset P. Vaccination against Q fever. In: *Vaccines against Viral and Rickettsial Diseases in Man.* Washington, DC: Pan American Health Organization; 1966: 528–531. PAHO Science Publication 147.

122. Bell JF, Lackman DB, Meis A, Hadlow WJ. Recurrent reaction of site of Q fever vaccination in a sensitized person. *Mil Med.* 1964;129:591–595.

123. Benenson AS. Q fever vaccine: efficacy and present status. In: Smadel JE, ed. *Symposium on Q Fever.* Washington, DC: Walter Reed Army Institute of Medical Science; 1959. Publication 6, Government Printing Office.

124. Lackman D, Bell EJ, Bell JF, Pickens EG. Intradermal sensitivity testing in man with a purified vaccine for Q fever. *Am J Public Health.* 1962;52:87–93.

125. Ackland JR, Worswick DA, Marmion BP. Vaccine prophylaxis of Q fever: a follow-up study of the efficacy of Q-Vax (CSL) 1985–1990. *Med J Aust.* 1994;160:704–708.

126. Genig VA. A live vaccine 1-M-44 against Q fever for oral use. *J Hyg Epidemiol Microbiol Immunol.* 1968;12:265–273.

127. Johnson JW, McLeod CG, Stookey JL, Higbee GA, Pedersen CE Jr. Lesions in guinea pigs infected with *Coxiella burnetii* strain M-44. *J Infect Dis.* 1977;135:995–998.

128. Waag D, Chulay J, Marrie T, England M, Williams J. Validation of an enzyme immunoassay for serodiagnosis of acute Q fever. *Eur J Clin Microbiol Infect Dis.* 1995;14:421–427.

129. Williams JC, Cantrell JL. Biological and immunological properties of *Coxiella burnetii* vaccines in C57BL/10ScN endotoxin-nonresponder mice. *Infect Immun.* 1982;35:1091–1102.

130. Williams JC, Damrow TA, Waag DM, Amano K. Characterization of a phase I *Coxiella burnetii* chloroform-methanol residue vaccine that induces active immunity against Q fever in C57BL/10 ScN mice. *Infect Immun.* 1986;51:851–858.

131. Brooks DL, Ermel RW, Franti CE, et al. Q fever vaccination of sheep: challenge of immunity in ewes. *Am J Vet Res.* 1986;47:1235–1238.

132. Waag DM, England MJ, Pitt ML. Comparative efficacy of a *Coxiella burnetii* chloroform:methanol residue (CMR) vaccine and a licensed cellular vaccine (Q-Vax) in rodents challenged by aerosol. *Vaccine.* 1997;15:1779–1783.

133. Waag DM, England MJ, Tammariello RF, et al. Comparative efficacy and immunogenicity of Q fever chloroform: methanol residue (CMR) and phase I cellular (Q-Vax) vaccines in cynomolgus monkeys challenged by aerosol. *Vaccine.* 2002;20:2623–2634.