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Q Fever: A troubling disease and a challenging diagnosis

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Abstract

Q fever is a disease caused by the bacterial pathogen *Coxiella burnetii*. This hardy organism can easily spread long distances in the wind, and only a few infectious aerosolized particles are necessary to cause serious illness. Presentations of Q fever disease can be wide-ranging, allowing it to masquerade as other illnesses and highlight the importance of laboratory testing for diagnosis and treatment. This review summarizes Q fever's epidemiology and clinical presentations and presents classical laboratory diagnostic assays and novel approaches to detecting this troubling disease.

EPIDEMIOLOGY

History

Coxiella burnetii is a bacterial organism that causes the disease Q fever. It was first identified by researchers in the United States and Australia in the 1930's [1, 2]. The discovery in the United States was somewhat serendipitous, as researchers working at the Rocky Mountain Laboratories in Hamilton, MT were studying the cause of Rocky Mountain spotted fever (RMSF). RMSF was known to be a tickborne disease, and the researchers were looking for the agent in pools of ticks collected near Nine Mile Creek west of Missoula, MT. The agent for RMSF was not found in these pools, but the studies did result in the identification of another pathogenic agent in a pool of 50 *Dermacentor andersoni* ticks – referred to as the "Nine Mile" agent [1]. This organism caused a febrile illness in guinea pigs and was first cultivated by Herald Cox at the Rocky Mountain laboratories [1]. Also, in the late 1930's, scientists in Queensland, Australia observed a new disease in slaughterhouse workers that they called "Q" fever [3]. Derrick and Burnet isolated a unique organism from these workers and characterized its infection in guinea pigs, rabbits, and mice [2]. In 1938, Rolla Dyer, who was the Chief of the Division of Infectious Diseases at the U.S. National Institutes of Health, traveled to Hamilton, MT, and handled guinea pigs and egg cultures infected with the "Nine Mile" agent. On his return from Montana, he became ill and isolated a bacterial

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agent from his own blood [1]. Further study and collaboration with the Australian group determined that the three bacterial agents, the Nine Mile agent, the agent of Australian "Q" fever, and the agent from Dyer's blood were all very similar. They all caused a similar illness in guinea pigs, and infection with one agent protected guinea pigs from infection with the other two agents [1]. Eventually, the agent was named *Coxiella burnetii* in honor of the American and Australian scientists.

Bacterial Characteristics

C. burnetii is a Gram-negative bacterium originally thought to be in the Rickettsiales order due to similar shared features, including its Gram staining, intracellular localization in arthropods, small size, and staining with aniline dyes [1]. However, detailed genetic analysis has shown that *C. burnetii* is a gammaproteobacteria and not an alphaproteobacteria like the Rickettsiales [4]. *C. burnetii* is genetically closely related to *Legionella pneumophila*, the cause of Legionnaires' disease, and the genome of *C. burnetii* consists of a single chromosome of approximately 2 million base pairs [4, 5]. All isolates of *C. burnetii* have nine conserved predicted ORFs either maintained on one of four plasmid types or in the case of plasmid-less strains; these sequences are incorporated into the chromosome [6]. Although functional roles have yet to be fully characterized, their conservation across all isolates of *C. burnetii* suggests a critically important role in survival. The genetic diversity of the remaining genes amongst the different plasmid types is also believed to play an important role in pathogenesis; however, this has yet to be fully elucidated. From its discovery until 2009, *C. burnetii* was only cultured in chicken eggs or within cultured host cells. In 2009, a culture media was described to support *C. burnetii* growth without a host cell [7]. Although this technique has opened many possibilities for researchers, *C. burnetii* is still considered to be an obligate intracellular organism in nature.

Reservoirs and Transmission

Although *C. burnetii* was originally cultured from ticks and can be found in contemporary tick samples, Q fever is generally not considered to be a tickborne disease. Transmission of *C. burnetii* to humans via tick bite seems to be quite rare. The common presence of *Coxiella*-like endosymbionts in ticks may cause an overestimate of the prevalence of *C. burnetii* in ticks [8]. The most common method of transmission is by inhalation of aerosols contaminated with the bacterium. *C. burnetii* can replicate in a wide variety of animal hosts, and human cases of Q fever have been associated with dogs [9], cats [10], and even three-toed sloths [11]. The most common reservoir hosts for *C. burnetii* are domesticated ruminants- sheep, goats, and cattle [12]. These animals can shed *C. burnetii* in urine, feces, vaginal mucus, semen, and milk [13]. *C. burnetii* can also infect trophoblast cells of the placenta and replicate to high densities in the placenta of ruminants and other mammals [14]. Although cattle can carry the fetus to full-term under these conditions, abortion of the pregnancy is common in sheep and goats. When infected ruminants give birth to viable or non-viable offspring, large amounts of *C. burnetii* can be released into the environment from the placenta and birth fluids. *C. burnetii* shed in this manner can either directly infect people at the time of birth or can contaminate the environment, whereupon bacteria-containing particles such as dust can be inhaled and result in infection. Infections due to materials being

carried by the wind have been reported to happen as much as 11 miles (18 km) from the point of origin [15].

Significance of Infected Reservoirs

The largest outbreak of Q fever was reported between 2007 and 2010 in the Netherlands [16]. Over 4,000 cases were reported to the public health authorities, but subsequent serologic testing suggested that at least 40,000 people were infected [17]. Most infections in this region took place via inhalation of organisms shed from infected goats. The infection of pregnant goats with *C. burnetii* resulted in the release of large numbers of bacteria into the environment at parturition. Many of the goats were unable to give birth to viable offspring due to the high bacterial load in the placenta. In retrospect, the increased numbers of abortions and stillbirths in the goat population were an early warning sign for this outbreak [16]. The resulting heavy bacterial burden after the goats gave birth resulted in human infections not only on farms but also in nearby villages. The most important risk factor for infection was living within 3 miles (5 km) of an infected dairy goat farm [16]. The outbreak was ended by implementing mandatory vaccination of goats and sheep against Q fever, and the culling of pregnant animals on infected farms [16].

Human Disease Surveillance

Several countries treat Q fever as a nationally notifiable condition. In the United States, cases have been reported to CDC since 1999. The number of cases reported each year between 2016 and 2018 has varied between 164 and 215. In Australia, 6,591 cases were reported between 2003 and 2017, or about 400-600 each year [18]. In France, from 2016 through 2018, between 172 and 251 confirmed cases were reported. The European Union reported between 922 and 1,058 confirmed cases per year during this time period, with France and Spain having the highest numbers [19]. There is some evidence that Q fever cases are underreported in these countries. For example, a serosurvey for Q fever on samples collected between 2003-2004 reported that nationwide seroprevalence for Q fever in the United States was 3.1% [20]. Even if antibodies persist for life, this seroprevalence rate suggests a much higher infection rate than is currently reported and suggests that a proportion of these infections are potentially asymptomatic or not diagnosed accurately. Similarly, 5.6% of Australian residents were seropositive in samples taken in 2012-2013 [21]. It is therefore suspected that the cases detected and reported are a small fraction of the actual number of *C. burnetii* infections.

Reservoir Surveillance

The presence of *C. burnetii* in livestock has been well documented. A survey of cow's milk in the U.S. found that 94% of bulk tank milk samples are positive for *C. burnetii* DNA using PCR [22]. At the level of the individual animal, 10.8% of cattle in the Republic of Korea and 17.7% of cattle in India were found to have antibodies against *C. burnetii* [23, 24]. A meta-analysis of bulk tank milk sampling from dairy cattle estimated overall herd-level prevalence of *C. burnetii* infection at 37% with a range of 10.7-76.9% [25]. In Germany, 2.24% and 3.58% of sheep and goats sampled in 2017-2018 had antibodies against *C. burnetii*, respectively [26]. Numerous studies have been performed and have found a range of seroprevalence in livestock, depending on the location and the species

sampled. A common theme in these studies is that livestock animals always carry some burden of *C. burnetii* infection, and in many cases the prevalence is quite high.

Occupational Risk

Because of the prevalence of *C. burnetii* in livestock hosts, Q fever is often thought to be an occupational disease, where jobs that involve contact with livestock put people at the greatest risk for Q fever. This concept is supported by numerous serosurveys of populations that have known occupational contact with livestock. A serosurvey of U.S. veterinarians found that 22% of those tested were positive for antibodies against *C. burnetii* [27]. Serologic tests in Germany found that shepherds and veterinarians had a seroprevalence of 77% and 64%, respectively [28]. In Italy, a group of veterinarians, slaughterhouse workers, and livestock handlers were tested and in 2015, with 63% of them were reported to be seropositive [29]. These examples are representative of numerous other studies showing very high *C. burnetii* seroprevalence rates among those who have livestock exposure. The actual percentages vary depending on location, with 12% of veterinarians in Sweden positive in a study from 1993 [30], and 83.7% of veterinarians positive in a study from the Netherlands in 1983 [31]. These differences may reflect differences in animal husbandry, use of PPE, prevalence in the livestock, or differences in the assays used to detect the antibodies. Nevertheless, it is clear from these studies that occupational exposure can result in much higher prevalence of infection compared to the general population.

Bioweapon

After World War II, the potential for the use of *C. burnetii* as a bioweapon was explored by several countries, including the U.S. and Soviet Union [32]. *C. burnetii* has properties that could make it an effective bioweapon, and it has been weaponized in the past. The first favorable property for use in biowarfare is its low infectious dose. Experiments performed in the 1950's using guinea pigs and human subjects demonstrated that the dose of *C. burnetii* needed to be inhaled for initiation of infection was less than 10 organisms [33]. Further analysis of these data suggested that the probability of inhalation of a single organism resulting in human infection was 0.44, while the probability that inhalation of a single organism resulted in illness was 0.12 [34]. It is not clear if all strains of *C. burnetii* have the same dose-response. It has been reported that there are significant differences in virulence of different strains in guinea pigs [35, 36].

The second feature of *C. burnetii* that causes concern for weaponization is its remarkable durability. When *C. burnetii* is outside of a host cell it assumes a dense, spore-like form known as the small cell variant (SCV) [37]. This form is a non-replicative form that can survive in nature for long periods. When the SCV enters a host cell, exposure to the acidic environment within the phagolysosome results in conversion of the organisms to the large cell variant [38]. This morphological form is metabolically active and produces gene products that subvert the phagolysosome for the purpose of supporting *C. burnetii* replication [39]. Remarkably, *C. burnetii* grows best at a pH of 4.5-5.0, which is the natural pH of the human phagolysosomal compartment. When *C. burnetii* are no longer able to continue replication, the organisms will revert back to the SCV, metabolic activity is suspended, and the organisms enter a dormant phase [38]. The SCV form of *C. burnetii* has

high resistance to heat (can survive brief incubations of 63°C), desiccation, and other stress [40]. Because *C. burnetii* is often present in cow's milk, temperatures for pasteurization have been set so that they are hot enough to kill *C. burnetii* [40]. In the U.S., pasteurization is most often accomplished by heating to 72°C for 15 seconds. *C. burnetii* has also been found to survive in the environment for a year or more, even under dry conditions [41, 42]. There is therefore a risk of *C. burnetii* infection in spaces previously occupied by livestock or other animals, even if they have been absent for long periods of time. The SCV also has some resistance to disinfectants. A study published in 1990 found resistance of *C. burnetii* to bleach, Lysol, and Roccal [43]. The same study found *C. burnetii* susceptible to killing by 70% ethanol and 5% chloroform [43]. Subsequent studies have found that *C. burnetii* has some susceptibility to bleach, but is less susceptible than most bacteria [44].

The third property that makes *C. burnetii* a potential bioweapon is its transmission by inhalation. The ability to spread *C. burnetii* through the air creates the potential for infecting large populations in a short period of time by distributing the organisms in infectious aerosols over a large area. In 1975, the U.S. and other countries ended their biological weapons programs following enactment of the Biological Weapons Convention. Since that time, work efforts for *C. burnetii* has been targeted toward improvement of public health.

CLINICAL MANIFESTATIONS

Acute Q Fever

Q fever infection can manifest as acute (primary) or chronic (localized) infections. While up to 50% of primary infections are thought to be asymptomatic, symptomatic acute infections typically present as a non-descript febrile illness following a 2 to 3 week incubation period [45]. Acute infections may present as a severe headache that is often retroorbital and includes photophobia [46]. Additional symptoms include fever, chills, malaise, and myalgia. Fever typically lasts 1-2 weeks and can relapse for an additional 1-2 weeks [47]. In some cases, acute Q fever will develop into an atypical pneumonia. In addition to the flu-like symptoms, nonproductive cough and inspiratory crackles are common with Q fever pneumonia, an illness that can range from a mild subclinical infection to a severe illness [48]. While radiography is necessary to diagnose pneumonia, it cannot distinguish Q fever from other etiologies [49].

Another common presentation of acute Q fever is granulomatous hepatitis; symptoms of which include fever, abdominal pain, nausea, and occasionally vomiting. Hepatic enzymes will be elevated 2 to 3 time normal, but jaundice is not common [50]. Myocarditis and pericarditis due to *C. burnetii* have been reported, but remain rare, representing <1% of acute infections. These presentations are typically detected by T-wave changes on an electrocardiogram [47]. Left untreated, acute Q fever is often a self-limiting illness and mortality is less than 2%; however, 20% of acute Q fever patients in the Netherlands outbreak were hospitalized, underscoring the potential for serious illness [47, 49, 51].

Chronic Q Fever vs. Persistent Focalized Infections

A small percentage of primary Q fever infections will enter a latent phase and reemerge as a more severe and localized infection months or years later and can occur regardless of initial symptomology or disease severity during the acute illness [49, 52]. Historically these cases have been termed chronic Q fever, due to the prolonged, lingering nature of the infection and the fact that treatment typically requires months or years of antibiotic therapy. Recently, some researchers have argued that the term ‘chronic’ may not be the best descriptor for these infections, as it is generic, does not identify the loci of the infection, and not all cases fall neatly into the case definition of chronic Q fever. It has recently been proposed that the location of these infections must be identified in order to determine the treatment strategy that is best suited to the infection, and that the term ‘persistent focalized infection’ should be adopted in the place of ‘chronic’ Q fever [50]. However, “chronic Q fever” is still the most common term for these prolonged infections. It is helpful to define these infections by the location of infection, such as Q fever endocarditis, Q fever vascular infection, Q fever osteomyelitis, as appropriate.

Chronic Q Fever Presentations

Q fever endocarditis is the most common manifestation of chronic Q fever infections [49]. Approximately 60-70% of all chronic Q fever cases are identified as endocarditis, with the aortic and mitral valves being commonly involved. Symptoms of Q fever endocarditis include low-grade fever, malaise, weakness, fatigue, chills, weight loss, anorexia, and night sweats. Patients are typically found to have heart failure or valve dysfunction and can also have splenomegaly and hepatomegaly [53]. Most patients who develop Q fever endocarditis have been found to have preexisting heart valve disease, and prior valve replacements are a common location for focalized infections [45, 54]. Q fever endocarditis is progressive if left untreated and will relapse if antibiotic therapy is stopped prematurely [47]. The second most common type of chronic Q fever infections are vascular infections, which are primarily seen in patients with preexisting aneurysms and vascular grafts or prostheses [50]. Vascular infections are commonly located in the abdominal or thoracic aortas. Clinical symptoms can include fever, weight loss, and abdominal pain [47]. The symptoms are typically nonspecific, which makes the diagnosis difficult and leads to poor outcomes [55]. In rare cases, *C. burnetii* can spread from vascular infections and lead to development of psoas abscesses [56].

Other presentations include osteoarticular infections. These infections have been described in multiple locations, including prosthetic joints [57]. Osteomyelitis is a common presentation of chronic Q fever in children but is rarely seen in adults [58]. Chronic hepatitis has been reported after known cases of acute Q fever hepatitis [47]. Chronic fatigue syndrome (also known as post Q fever fatigue syndrome) has been reported following cases of acute Q fever; symptoms include prolonged fatigue, sweating, breathlessness, swollen lymph nodes, and blurred vision [47, 49, 50].

Imaging studies are important clinical tools for diagnosing endocarditis and vascular infections and is also helpful when locating the foci of infection for other manifestations of Q fever disease. Evidence of infection can be elucidated by computed tomography (CT)

scan, ultrasonography, magnetic resonance imaging (MRI), positron emission tomography (PET), and leukocyte scan [59]. Echocardiograms are employed to detect valvular lesions in patients with Q fever endocarditis, but due to their small size, vegetations are detected in only 12% of cases. A transesophageal echocardiogram can improve detection relative to the less sensitive transthoracic echocardiogram in prosthetic valve endocarditis [60]. The use of F-FDG PET/CT ^{18} has also been demonstrated to be an effective tool in diagnosing patients with chronic Q fever and during follow-up [61]. These imaging tools are important components for clinical diagnosis of chronic Q fever when interpreted in concert with laboratory diagnostic assays [49].

Treatment

It is not recommended to treat asymptomatic Q fever infections. For symptomatic acute Q fever, a 14-day course of doxycycline is preferred. Treatment is most effective when started within 3 days of symptom onset; therefore, it is recommended that treatment be started if Q fever is suspected rather than waiting for laboratory confirmation [46, 49]. For patients who cannot tolerate doxycycline, other tetracyclines or fluoroquinolones have proven effective in treating Q fever [50]. Doxycycline is contraindicated in pregnant women; therefore co-trimoxazole is typically prescribed [49]. Historically, doxycycline was also contraindicated for use in children under 8 years of age due to the risk of dental staining of adult teeth [58]. However, recent studies have shown that the risk of dental staining is low; thus doxycycline can safely be used in young children [62].

Like acute Q fever, chronic Q fever infections are treated with tetracyclines. For Q fever endocarditis, the preferred course of treatment is doxycycline and hydroxychloroquine for 12-18 months. Vascular infections are also treated with doxycycline and hydroxychloroquine; however, these infections require antibiotic therapy for a minimum for 24 months [50]. Any lapse in treatment will cause relapses of symptoms and progression of disease. For pregnant women diagnosed with chronic Q fever, the preferred treatment is co-trimoxazole during pregnancy and then doxycycline with hydroxychloroquine once the patient has given birth [49, 63]. For children with Q fever endocarditis, surgery is recommended in addition to a regimen of doxycycline and hydroxychloroquine. For children diagnosed with Q fever osteomyelitis, variable combinations of co-trimoxazole, ciprofloxacin, rifampin, doxycycline, clarithromycin, and azithromycin have been used, along with surgical drainage or debridement of the infected region [50].

LABORATORY DIAGNOSTICS FOR Q FEVER

Laboratory confirmed diagnosis of Q fever is primarily achieved through serology. Antigens used in serological assays are typically inactivated whole cells from two antigenically discrete strains of *C. burnetii* termed phase I and phase II. Nine Mile phase I (RSA 493) is the *C. burnetii* reference strain [47]. The Nine Mile phase II clone 4 strain of *C. burnetii* is an avirulent form of the phase I strain. Generated from serial passages of phase I in embryonated eggs, the phase II antigen is unique from its parental strain through acquired genetic mutations. A chromosomal deletion of approximately 26 kb has resulted in the loss of multiple lipopolysaccharide (LPS) biosynthesis genes [64]. This deletion renders the

phase II strain incapable of producing full-length LPS. It lacks some core sugars and the O-antigen, which is believed to be a predominant antigen recognized by phase I-specific serum antibodies [65]. It has been suggested that detection of phase II specific antibodies is a result of the truncated LPS on the whole-cell phase II antigen, which allows antibodies access to antigens on the cell surface of the phase II strain that would otherwise be blocked by steric hinderance of the full-length LPS in the phase I strain [66]. Propagation of the antigen is carried out in either embryonated egg yolks or cell culture.

Acute Q fever often presents with higher levels of IgG antibodies against the phase II antigen [49]. Diagnosis of acute Q fever is often made based on a four-fold increase in IgG antibody titers against the phase II antigen between samples collected during the acute and convalescent stages of infection. Acute serum samples should be collected within one week of the onset of symptoms, and the convalescent sample should be collected 3-6 weeks later. In the absence of paired sera, a single sample with a phase II IgG titer ≥1:128 may indicate acute Q fever; however, a sample collected during the acute stage of illness may lead to a false negative result if tested in the absence of a convalescent sample because antibodies typically do not develop until 7 to 15 days post symptom onset [49]. By the third week of illness, 90% of patients will seroconvert, and phase II specific IgG antibodies typically peak at 8 weeks post symptom onset [49]. Antibodies against IgM are also commonly used for diagnosing Q fever; however, the specificity of IgM assays can be low due to the potential for cross-reactivity, which has been reported for *Legionella* and *Bartonella* species [49, 67, 68]. In a study of 19 acute Q fever patients, inclusion of IgA was shown to increase specificity from 87% with either IgM or IgA alone to 100% when used in combination; however, the ability to detect IgA in serum from acute Q fever patients is not consistent [69].

The hallmark of chronic Q fever is an elevated phase I antibody titer greater than or equal to the phase II antibody titer [49]. However, Q fever serology to distinguish chronic from acute infections should be interpreted with caution as some strains of *C. burnetii*, such as Guyana, have been shown to cause an elevated phase I titer during acute infection, relative to the phase II titer [70]. Furthermore, phase II titers exceeding levels of the phase I titers have been documented in some cases of chronic Q fever [71, 72]. Antibody titers may continue to rise and may remain detectable from months to years or persist for life after the infection regardless of treatment success or symptom status, which may confound test results. Early antibiotic treatment does not influence IgG antibody titers [49]. Q fever can also be diagnosed by PCR through detection of *C. burnetii* DNA, detection of *C. burnetii* antigens by immunohistochemistry, or isolation of bacteria by culture from clinical specimens; however, each method has different advantages as discussed below [49].

Indirect Detection Methods

Detection of antibodies against *C. burnetii* is an important diagnostic tool since patient history, clinical imaging studies, and symptomology are not sufficient for a definitive diagnosis of Q fever. Direct methods of detecting *C. burnetii* during infection are limited to early acute illness or involve invasive sample types from localized infections. Therefore, indirect methods for the diagnosis of Q fever are essential.

Indirect Immunofluorescence Antibody (IFA).—There are three commercially available serological assays for the diagnosis of Q fever. Of these, the gold standard is the indirect immunofluorescence antibody assay (IFA). To perform the IFA, phase I and phase II antigens are coated onto individual wells of a microscope slide. Serum samples are serially diluted, and antibodies are detected with a fluorescein isothiocyanate-labeled secondary antibody, which is visualized under a microscope. The presence of rheumatoid factor (RF) in the serum can lead to false-positive IgM results [73]. Screening for RF or use of a RF absorbent can be useful in these cases. Cross-reactions with egg antigens may also lead to false-positive results [73]. Determination of cut-off titer for background is variable and dependent on the testing laboratory or population being tested [73]. Limitations to this assay include the subjective nature of the visualization step, which can lead to inter-assay and inter-laboratory variability. Furthermore, different antigen preparations and assay protocols may influence end-point titers [49]. As such, acute and convalescent serum should be tested at the same time [49].

Complement fixation test (CFT).—Another commercially available test as a kit for detecting antibodies against *C. burnetii* in blood samples is the complement fixation test (CFT). Patient serum is subjected to heat to inactivate natural complement before testing. *C. burnetii* specific antibodies in the tested serum complex with the phase I or phase II antigen. This complex fixes the complement and inhibits lysis of sheep red blood cells (RBCs) in the assay. In the absence of anti-*C. burnetii* antibodies, complement is not fixed, and RBCs are lysed. This assay offers an economical option that is very specific; however, it lacks sensitivity [74]. In a study comparing the CFT to the IFA, which was shown to detect IgM antibodies as early as three days after symptom onset, the CFT assay could not detect IgM in any of the tested samples during the first week of symptom onset. Furthermore, antibody recognition by CFT was inferior to the IFA even at three weeks post symptom onset [74]. The time-consuming and labor-intensive aspects of this assay limit its usefulness.

Enzyme-linked immunosorbent assay (ELISA).—The third commercially available method of antibody detection for Q fever is the enzyme-linked immunosorbent assay (ELISA). Antigen is coated onto 96-well plates, which allows for high-throughput screening. Serum is added to the plate wells and any antigen-antibody complexes are detected with a labeled secondary antibody. The addition of a substrate allows for detection using a microplate reader. Comparison of the three assay types (IFA, CFT and ELISA), shows similar diagnostic efficacy within three months after symptom onset, but IFA performed better in follow-up serum [75]. IFA remains the most common diagnostic method for Q fever, but ELISA can be more useful in screening larger numbers of samples, such as in seroprevalence studies. The development of recombinant protein-based antigen to replace the whole cell *C. burnetii* antigen in ELISA assays have been investigated to eliminate culturing of large quantities of this select agent. Top recombinant antigens include Com1, YbgF, and GroEL and are highly specific, but lack sensitivity compared with the whole-cell antigen [76, 77]. Sensitivity and specificity for Com1 were reported to range from 55% to 37.5% and 90% to 71%, respectively, while the sensitivity of YbgF was reported to be 72% [76]. GroEL sensitivity reportedly ranged from 88% to 71% with 90% specificity [76, 77].

Therefore, laboratories considering such assays need to carefully validate their performance characteristics to accepted reference methods prior to implementation in the clinical setting.

Western blot (WB).—Western blot (WB) analysis can be performed to visualize the antibody response to Q fever and is useful in distinguishing between chronic and acute infections, as far more protein antigens are recognized by chronic Q fever serum [78]. Whole cell *C. burnetii* antigens are separated on a sodium dodecyl sulfate-polyacrylamide gel before transferring to a membrane. The membrane is incubated with serum and any *C. burnetii* specific antibodies present in the serum are detected with a labeled secondary antibody. Visualization can occur through a wide range of detection methods. In serum from chronic Q fever patients, antibodies typically react with proteins at 50-, 80- and 160-kDa [73]. WB is a sensitive test, but the specificity of this assay is controversial [73]. This assay is not ideal for screening large numbers of samples as it is time-consuming and labor-intensive.

Enzyme-linked Immunospot (ELISPOT).—The *Coxiella* ELISPOT assay was developed for the early diagnosis of chronic Q fever [79]. Unlike other serological assays previously discussed, which are a measure of humoral immunity, the ELISPOT assay measures *C. burnetii*-specific T-cell responses. Mononuclear cells are isolated from whole blood and stimulated with phase I and phase II antigens on PVDF strip plates. The secreted cytokine, interferon-gamma (IFN-γ), is captured with labeled anti-IFN-γ antibodies. A substrate is added to detect the antibody complex and the spots, which correspond with an individual T-cell producing IFN-γ, are enumerated using an ELISpot reader [79]. Patterns of T-cell responses in this assay following stimulation with phase I and phase II antigen are similar to the antibody responses observed for chronic vs. acute Q fever. An interferon-gamma response assay (IGRA) to measure cellular immunity to *C. burnetii* has been developed and was shown to be effective. The Q-detect test (Innatoss Laboratories BV, Netherlands) has a sensitivity of 93% and specificity of greater than 90% but is not currently available for human clinical diagnostic testing [80]. Advantages of the IGRA assay over the IFA include elimination of inter-assay and inter-laboratory variability [81]. However, the IGRA is more time-consuming, and unlike samples for serological testing, which can be stored frozen, whole blood for measurement of cellular immunity must be fresh (<12 hrs.), which limits its usefulness in certain diagnostic situations [81].

Direct Detection Methods

Direct methods for Q fever diagnosis are best utilized for patients with clinical evidence of acute Q fever within 14 days since symptom onset or for patients presenting with chronic Q fever [49]. This analysis is most useful either before or within 48 hours of antibiotic administration [49].

Molecular Methods.—Diagnostic testing to detect the presence of C. burnetii DNA is often achieved through PCR-based analysis, including conventional PCR, nested PCR, or real-time PCR [82]. The most commonly targeted gene, IS1111, is a multicopy repeated element, which allows for specific and sensitive detection of C. burnetii. Many different gene targets have been optimized with varying sensitivity and specificity, including com1,

icd, and ompA, among others [82]. PCR-based assays can be used to detect C. burnetii in a wide range of clinical samples, including blood, serum, cerebrospinal fluid, bone marrow, vascular aneurysm, and tissue biopsies [49]. Fresh, frozen, or even formalin-fixed tissues can be used [73]. PCR has been shown to be essential for diagnosis of Q fever in the first week after symptom onset, but sensitivity declines rapidly thereafter [49]. Negative results should be interpreted with caution as PCR inhibitors, reduced primer binding due to variability in the C. burnetii DNA, or C. burnetii present in quantities below the limit of detection can lead to false negatives [83].

Loop-mediated isothermal amplification (LAMP) assays are a unique method of DNA detection for Q fever diagnosis [84, 85]. This method eliminates the need for specialized equipment by employing a *Bst* DNA polymerase, which allows strand displacement-DNA synthesis for amplification at a single temperature. Relative to real-time PCR, this technique was found to be more sensitive (33% vs 8.3% for 24 acute Q fever samples) and rapid (1 hr.). Another unique method for detection of *C. burnetii* DNA in blood plasma was developed by Koo *et al.*, using silicon microring resonator [86]. DNA is amplified under isothermal conditions (37 to 42°C) using a recombinase polymerase amplification solution for this assay. *C. burnetii* in the samples is detected by a measurable shift in the resonant wavelength due to binding of the amplified DNA to an immobilized primer. Among 16 acute Q fever patients and 19 patients with other febrile illnesses, reported sensitivity and specificity were 87.5% and 89.5%, respectively. This method is label-free and can be completed in as little as 10 minutes; however, it does require the use of specialized equipment.

A next-generation sequencing (NGS)-based approach to identifying a wide range of microbial DNA at one time has been developed. This assay is useful for Q fever diagnosis, given the non-specific disease symptoms that often accompany this disease. The assay allows for rapid screening of numerous pathogens from a plasma sample, which may provide earlier diagnosis and reduce the need for tissue biopsy. Kondo *et al.*, reported the usefulness of this method in diagnosing and genotyping *C. burnetii* in a patient with chronic Q fever endocarditis [87]. Microarray-based assays are also useful for detection of *C. burnetii* [82]. These assays are highly specific and have similar detection limits to conventional PCR assays. The benefits of the microarray-based assay are the ability to multiplex gene targets and improve detection of novel species or variants.

Image-based Assays

Immunohistochemistry (IHC) is a diagnostic tool useful for detecting *C. burnetii* in tissues of Q fever patients with localized infections. Samples can be tested fresh or from formalin-fixed paraffin-embedded tissues. Tissue sections are prepared on slides, and *C. burnetii* is distinguished with an anti-*C. burnetii* monoclonal antibody. The complex is then detected by a labeled secondary antibody and visualized under a microscope. Fluorescence *in situ* hybridization (FISH) is a similar but more sensitive technique that has been used for the diagnosis of chronic Q fever endocarditis and vascular infections [82]. Through this technique, *C. burnetii* DNA is detected in tissue sections using a fluorescently tagged oligonucleotide probe (targeting 16S rRNA). Peptide nucleic acid (PNA) probes, which are

synthetic homologs of nucleic acids, allow for faster and more sensitive analysis than with standard oligonucleotide probes, albeit at an increased cost [88]. A pan-bacterial probe can be employed as well, and, in these cases, infection by *C. burnetii* can be confirmed by PCR and sequencing [89].

Culture

Propagation of *C. burnetii* is restricted to select BSL3 facilities due to its categorization as a potential bioweapon and select agent; therefore, culturing is not routinely performed in clinical labs or for diagnostic purposes [90]. *C. burnetii* can be propagated in a variety of cell types, including macrophages (DH82, P388D1, J774), fibroblasts (L929, HEL), and epithelial cell lines (RK13, Vero E6). With the development of acidified citrate cysteine medium (ACCM), growth of *C. burnetii* is no longer restricted to the intracellular environment [7]. Isolation from clinical samples has been demonstrated for the second-generation formulation (ACCM-2); however, despite having been refined into a third generation (ACCM-D) formulation, isolation of some clinically relevant strains of *C. burnetii* may not supported by this media [82]. Additionally, the administration of antibiotics for treatment and slow growth kinetics can hinder culturing success. While *in vitro* isolation is possible in limited situations, it is not the method of choice for routine diagnostics.

CONCLUSION

Since its discovery in the 1930s, significant advancements have been made towards understanding Q fever disease and its diagnosis, yet many challenges remain. Nonspecific disease symptoms coupled with long-lasting antibodies and a complicated interpretation of differential reactivity to the two antigenic phases of *C. burnetii* are a few of the obstacles that remain. Despite decades of research towards the development of new tools and techniques, the IFA remains the reference method for diagnosing Q fever. Future research towards improved methodologies that differentiate acute, chronic, and past infections and methods that eliminate the inherent variability of the IFA test will be important tools in the fight against this troubling disease.

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Table 1.

Summary of methods for Q fever diagnosis

Method	Advantages	Limitations
Indirect Detection Methods		
Indirect Immunofluorescence Assay (IFA)	specific, sensitive, economical	subjective, inter-assay and inter-laboratory variability, convalescent sample needed for confirmation
Complement Fixation Test (CFT)	specific, economical	time-consuming, labor intensive, lacks sensitivity, convalescent sample needed for confirmation
Enzyme-linked immunosorbant assay (ELISA)	sensitive, high throughput	difficult to interpret results, inferior to IFA in follow-up serum
Western blot (WB)	ability to differentiate reactive antigens	time-consuming, labor intensive, not suitable for large sample sizes
Enzyme-linked immunospot (ELISPOT)	reduced inter-assay and inter-laboratory variability relative to IFA	time-consuming, requires fresh (<12 h) samples
Direct Detection Methods		
PCR (conventional, nested, real-time)	broad specimen range, specific, sensitive, economical	requires specialized equipment, sensitivity declines after first week of symptom onset, possibility of false negatives
Loop-mediated isothermal amplification (LAMP)	eliminates need for specialized equipment, sensitive, rapid	limited use in clinical setting
Silicon microring resonator	label-free, rapid	requires specialized equipment, limited use in clinical setting
Next-generation sequencing (NGS)	screen for multiple pathogens simultaneously, multiplex targets, detect novel species/variants	requires specialized equipment, time-consuming, labor intensive, sensitivity declines after first week of symptoms
Immunohistochemistry (IHC)	rapid direct detection without the risk of working with viable organism	requires specialized equipment, difficult to quantify, requires biopsy
Fluorescence in situ hybridization (FISH)	more sensitive than IHC	requires specialized equipment, difficult to quantify
Culture	direct pathogen detection, allows for cultivation/characterization of isolates	hazardous, time-consuming, restricted to select facilities, may need to confirm by PCR/other method