Future MICROBIOLOGY

Review

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# Q fever – immune responses and novel vaccine strategies

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Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*. It is an occupational risk for employees of animal industries and is associated with contact with wildlife and domestic animals. Although Q fever infection may be asymptomatic, chronic sequelae such as endocarditis occur in 5% of symptomatic individuals. Disease outcomes may be predicted through measurement of immune correlates. Vaccination is the most efficient method to prevent Q fever. Currently, Q-VAX is the only licenced human vaccine. Q-VAX is highly effective; however, individuals previously exposed to *C. burnetii* are at risk of adverse reactions. This review examines the immunological responses of acute and chronic Q fever and the efforts to provide a safer and cost-effective Q fever vaccine.

**Plain language summary:** Q fever is a disease that is spread by some animals, such as sheep and cattle, to humans. Although most people will recover if they get Q fever, some become very ill. There is a vaccine for Q fever (Q-VAX), but it can cause a reaction when given to some people. Research is ongoing into how the human immune system reacts to the bacteria that causes Q fever. A small number of people who get Q fever will develop a prolonged disease that can be serious and affect the heart, which is why there is also research into developing new vaccines for this disease. This research will look at those parts of the germ that causes Q fever that can be used for a new vaccine.

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**Keywords:** *Coxiella burnetii* • immune responses • immunisation • Q fever • Q-VAX • vaccination

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*, that can be found worldwide except for New Zealand and French Polynesia [1,2]. The disease was first described as a febrile illness spreading among workers in an abattoir in Queensland, Australia, in the early 1930s [3] (Figure 1). As the causative agent was not identified at the time, it was labeled Q fever for 'query' fever [3]. Samples from these cases were analyzed by Burnet and Freeman in Melbourne, Australia, where an intracellular bacterium was isolated [4]. Coincidently in the USA, Davis and Cox identified a previously unknown bacterium in ticks [5]. Comparisons of the clinical responses in those infected with the organisms were found to be similar, leading to the discovery that these were the same organism [6]. As the organism displayed rickettsia-like properties it was originally named *Rickettsia burnetii*; however, it was eventually renamed *Coxiella burnetii* in honor of those who had first described the bacterium. Many years later, genomic sequencing would in fact reveal that *C. burnetii* was distinct from the Rickettsia group [7].

Q fever is most commonly associated with occupations that involve close contact with animals. Cattle, sheep and goats were traditionally regarded as the primary reservoirs for infection; however, it is now recognized that the bacterium may be carried by many animals including wildlife, feral animals and domestic pets [8]. Accordingly, the range of occupations with a high risk of exposure to *C. burnetii* is extensive including abattoir workers, farmers, shearers, veterinarians and their staff, wildlife and zoo workers, cat and dog breeders and research laboratory personnel. Furthermore, seroprevalence data suggests that simply living in a rural area may increase the risk of infection [9,10]. Primarily, transmission to humans is via inhalation of airborne *C. burnetii* contaminated dust

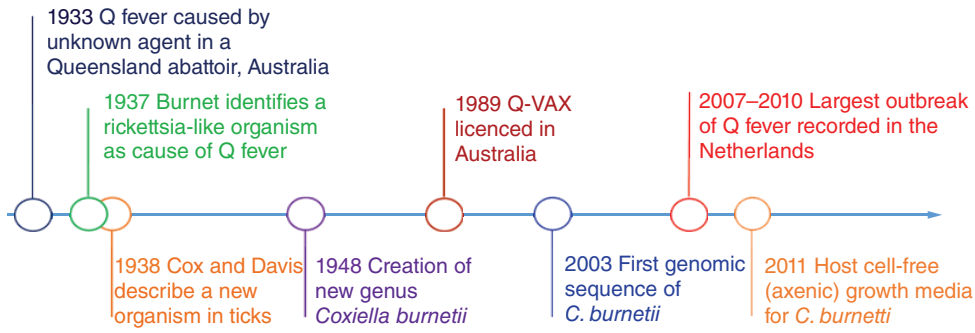


**Figure 1. Landmark events in Q fever research.**

particles that can travel long distances, although contact with blood, tissue and birthing fluids from infected animals is also hazardous [11].

Protective barrier methods such as respiratory protective masks or respirators would provide some protection but would be of little practical use to individuals exposed outside of the traditional occupational risk groups. Farm animals can also be vaccinated before exposure to *C. burnetti*. However, the currently available veterinary whole cell vaccines against coxiellosis have mostly failed to protect transmission due to ineffective implementation and questionable efficacy of these vaccines in veterinary practice. Moreover, most Q fever acquired in Australia is now largely in the nonoccupational risk groups, suggesting that this would not be practical. Due to the highly resistant nature of *C. burnetii* and its capacity to cause infection, prevention of Q fever is primarily afforded by vaccination. Currently, there is only one licenced Q fever vaccine for human use. Q-VAX (Seqirus, Parkville, Australia) was licenced in Australia in 1989; however, it requires prevaccination screening to avoid adverse reactions in individuals who have been previously exposed to *C. burnetii*. This process is time-consuming, costly and an impediment to large-scale vaccination programs. Therefore, development of a new safe and effective Q fever vaccine would be beneficial.

## The bacterium

*C. burnetii* is an obligate intracellular Gram-negative bacterium. It demonstrates remarkable resistance to physical and chemical stressors which can be attributed to its two morphological forms: the small cell variant (SCV) and large cell variant (LCV) [12]. As an SCV, it can survive harsh external environmental conditions as well as the acidic conditions of a phagolysosome, where it can survive and transform into the metabolically active and replicative LCV [12]. *C. burnetii* also undergoes antigenic phase variation. The virulent phase 1 (Ph1) bacterium is highly infectious and possesses a smooth, full-length lipopolysaccharide (LPS) in its cell wall that is thought to be crucial in establishing infection [13]. In contrast, the Ph 2 *C. burnetii* LPS is 'rough' and truncated, lacking an O-antigen, and is considered relatively avirulent [13]. Intra- and inter-strain variation in LPS has also been shown to have a significant effect on bacterial virulence [14]. Importantly, this antigenic phase variation exhibited by *C. burnetii* is useful in serological diagnosis because it can be used to distinguish between acute and chronic disease, with acute Q fever usually characterized by higher Ph 2 antibodies and chronic Q fever usually having higher Ph 1 antibodies.

## Clinical presentation of Q fever

Infection may result from exposure to only a few *C. burnetii* organisms [15]. An incubation period of 15–25 days allows the bacteria to multiply and establish infection [4]. The course of infection with *C. burnetii* varies between individuals. Many people infected with *C. burnetii* may be asymptomatic or have mild symptoms and often remain undiagnosed [16]. Symptomatic acute Q fever can occur in approximately 40% of infected individuals, although in some outbreaks a higher incidence has been reported [17]. Symptoms may include fever, rigors or chills, headaches, flulike symptoms, acute fatigue and possible manifestations of pneumonia, hepatitis and cardiac infections [18]. A review of patients with Q fever from North Queensland, Australia, showed that the most frequent presentation of acute Q fever was with fever, nonspecific musculoskeletal symptoms such as myalgia, arthralgia and gastrointestinal symptoms such as nausea and vomiting [19]. Recovery is expected for most patients with acute Q fever, although some may experience ongoing headaches, muscle and joint pain, sweats and fatigue up to 12 months following the initial infection [4]. Ongoing symptoms are usually due to post-Q fever fatigue syndrome (QFS; discussed subsequently).

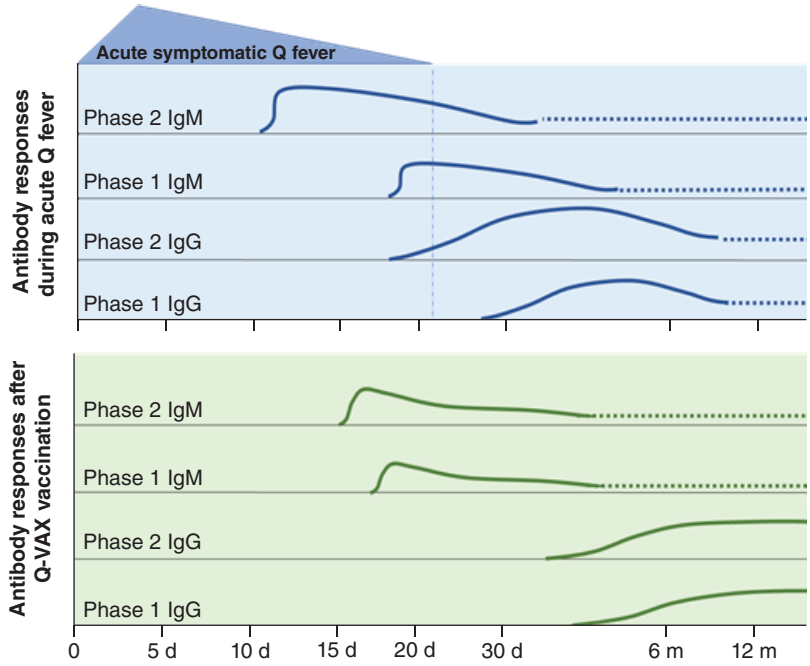


**Figure 2. Typical serological responses in acute Q fever and in vaccines.** Various diagnostic tests may be used for the diagnosis of Q fever. Although PCR can detect *Coxiella burnetii* in the early stages of infection, most cases are detected using serological methods such as IFA, CFT and EIA. IFA is the gold standard reference method for *C. burnetii* antibody detection. IgM and then IgG antibodies develop to Ph 2 antigens in 10–14 days from symptom onset. Seroconversion or a fourfold rise in Ph 2 IgG titer is diagnostic of acute Q fever.
CFT: Complement fixation test; EIA: Enzyme immunoassay; IFA: Immunofluorescence assay; Ph: Phase.
By IFA. Figure reproduced with permission from 'Q fever queries and answers', Chaudhuri and Robson (2013).


The diagnosis of acute Q fever is based on evaluation of patient history and risk factors for exposure but must also be confirmed with laboratory evidence. Although any detection of *C. burnetii* by PCR provides a definitive diagnosis of either acute or chronic Q fever, serological testing remains the traditional gold standard for diagnosis of Q fever [20]. Paired serum samples should be collected 2–3 weeks apart to determine any changes in the antibody titer. Antibodies can be detected from ~10 days after onset of symptoms [4]. Initially, IgM antibodies against Ph 2 antigen can be detected in serum, followed by IgM antibodies to Ph 1 antigen. In the subsequent weeks, IgG antibodies against Ph 2 and then Ph 1 antigen begin to increase [4]. Acute Q fever characteristically has higher IgG antibody titers against Ph 2 than Ph 1 *C. burnetii* (Figure 2).

Up to 5% of those infected with *C. burnetii* may develop 'chronic' Q fever or as has been suggested as an alternative term, a focal and persisting form of the disease, several months or years after the initial infection [21]. Certain predisposing conditions, such as pre-existing cardiac valvular disease, immunosuppression or pregnancy, can increase the risk of developing chronic Q fever [22]. Clinically, chronic Q fever symptoms may be variable. Q fever endocarditis is the most common manifestation and, if left untreated, may be fatal [23]. Other complications include vascular infections, osteomyelitis and hepatitis [22]. Diagnosis is most commonly by serological evidence because culturing of *C. burnetii* is difficult and PCR detection of *C. burnetii* DNA in blood has low sensitivity [24], although high sensitivity in surgically removed cardiac valves. Serologically, chronic Q fever is characterized by high Ph 1 IgG antibody titers that do not fall with time [21], and although it is not considered diagnostic, high levels of Ph 1 IgA antibodies may also be present [2,25]. Recently, the use of the term 'chronic Q fever' has become controversial along with the level of the elevated Ph 1 titer required to support the diagnosis. It is suggested that greater emphasis should be placed on clinical and relevant imaging findings rather than an elevated Ph 1 IgG level [26].

QFS is another clinical outcome of *C. burnetii* infection. A study by Wildman and colleagues reported that approximately 20% of acute Q fever patients develop QFS [27]. It has been described as a debilitating and long-term fatigue, which may persist for up to 10 years. Some doctors confuse 'chronic' Q fever with the post-QFS because symptom profiles overlap, especially with respect to fatigue. However, these latter patients show no signs of

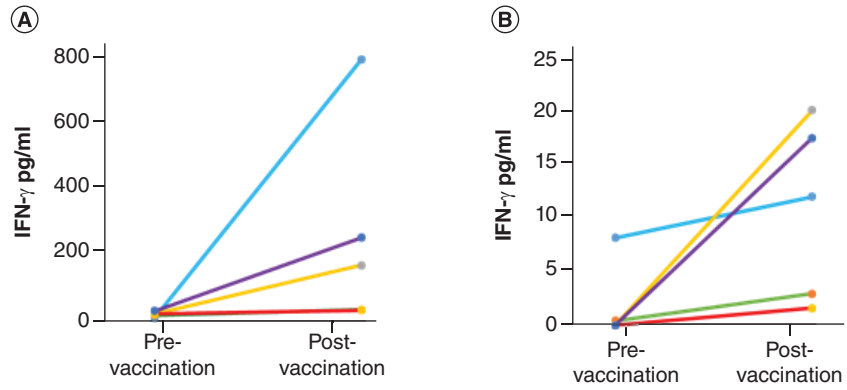


**Figure 3. Production of IFN-γ 4 weeks post-Q-VAX.** Assessment of IFN-γ is an indirect measure of the development of post-vaccination specific T-cell mediated immunity to Q-VAX antigen. Supernatants from **(A)** whole blood from volunteers (n = 5) receiving Q-VAX and **(B)** mononuclear cells separated from the same volunteers (n = 5) was incubated with Q-VAX in culture before and 4 weeks after vaccination were assessed for IFN-γ using ELISA. In postvaccine samples, the levels of IFN-γ was significantly higher compared with those before vaccination, indicating the generation of memory T cells following vaccination (unpublished data – Ketheesan N *et al.*).

focalized chronic infection (i.e., no endocarditis). They appear to have an immunological abnormality involving an oversensitive cell-mediated immune responses and dysregulated cytokine production [28].

## Immune responses in Q fever

The complex nature of the immune responses has now become clearer with studies targeting specific host responses. Both humoral and cell-mediated immune responses play an important role in the control of infection with *C. burnetii*. It has been shown that adoptive transfer of immune sera or splenocytes mediated protection in naive mice [29]. Passive transfer of immune serum into recipient mice inhibit the growth of the bacterium, and thus T cells were required to control the infection. Transfer of T cells conferred significant protection in SCID mice highlighting the critical role of these cells in controlling *C. burnetii* infection.

The immune status of the host can contribute to the overall disease outcome of infection with *C. burnetii*. Helbig and colleagues [30] compared the frequencies of allelic polymorphisms in three Q-fever patient groups. In patients with Q fever endocarditis, differences in the IL-10 promoter microsatellites R and G was observed with higher frequencies of the TNF-α receptor II 196R polymorphism. There were no significant differences in allelic frequencies between the control group and the patients who had acute uncomplicated Q fever. These observations highlight that the immune status of the host is due to the variations in immune responses associated with immunogenetic differences of the host, rather than the characteristics of *C. burnetii*, mainly influenced the disease process and severity [30].

Impaired cell-mediated immune responses lead to chronic Q fever with endocarditis with persistence of *C. burnetii*. A study in 2012 [31] found that patients with Q fever endocarditis had significantly increased Tregs, characterized by the expression of CD25 and Foxp3, compared with healthy controls. The investigators proposed that Tregs suppress effector T-cell activity, thereby preventing the removal of *C. burnetii*, leading to the development of chronic Q fever [31].

*C. burnetii* has been found to alter the immune response of infected individuals [32]. *C. burnetii* stimulates production of IL-10, which deactivates monocytes through suppression of TNF-α. Patients with Q fever endocarditis have been found to produce increased TNF-α in monocytes; however, studies disagree on the impact of high TNF-α upon *C. burnetii* replication. It has been observed that levels of TNF-α and IL-10 are increased in cases of valvopathy and endocarditis. Additionally, the B cell marker sCD23 has been observed to be significantly increased in Q fever endocarditis patients in comparison to acute Q fever patients and controls. Therefore, sCD23 has been proposed as a potential marker for Q fever endocarditis. Similarly, research has explored measurement of cytokines in serum as a diagnostic tool for differing manifestations of Q fever. IFN-γ/IL-2 ratios >11 have been found to be strongly indicative of chronic Q fever. In addition to differentiation of acute, chronic and past Q fever, studies have found IFN-γ production in response to *C. burnetii* antigens to be a potential indicator for post-vaccination immunity (Figure 3) [33].

In a recent study [34], it was shown that in the replication and dissemination of *C. burnetii* syntaxin 11 (STX11) played a critical role as a restriction factor that inhibits *C. burnetii* intracellular replication. It has also been known that IFN-γ restrict *C. burnetii* replication within cells by restriction of replication of the bacterium that is mediated by Indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme that depletes tryptophan in the host cell and deprives the bacterium of nutrients [34]. Host cells deficient in IDO1 activity, in the presence of IFN-γ are still able to restrict *C. burnetii* replication, which suggest several mechanisms involved in host defense against *C. burnetii*. Along with IFN-γ, STX11 has been found to contribute to suppression of *C. burnetii* replication. STX11 mediates fusion of host vesicles with specific subcellular organelles. Depletion of STX11 has been shown to enhance intracellular replication of *C. burnetii* [34]. Further research into immune correlates of Q fever is therefore essential to develop vaccine strategies [33,35].

## Vaccination against *C. burnetii*

As noted earlier, *C. burnetii* is highly resistant and can survive in the environment for long periods [21]. Furthermore, its ability to spread as an airborne pathogen and its capacity to cause infection with only a few infectious particles makes infection control problematic. Vaccination is the most effective method for the prevention of Q fever.

### Historical vaccine development

Vaccine development against *C. burnetii* began soon after the pathogen's discovery (Table 1). Early whole-cell vaccines were prepared from infected yolk sacs after several consecutive passages [36]. In 1948, as cited in Benenson, Smadel *et al.* developed a vaccine from the Henzerling strain of *C. burnetii*, which was formalin killed and ether extracted [37]. This vaccine, which contained 10% yolk sac, was trialled in humans and found to be protective against Q fever for at least 11 months. However, adverse reactions at the injection site in individuals previously exposed to the bacteria, either naturally or following repeated immunizations, were reported [37]. In the former Soviet Union, both killed and live vaccines were used in mass immunization trials [38,39]. High reactivity encountered using whole-cell killed *C. burnetii* vaccines led to the development of the live attenuated M-44 vaccine [39]. This live vaccine was obtained after the 44th passage of the Grita strain of *C. burnetii*, and as Fiset suggested, this was most likely a Ph 2 vaccine [36,40]. Early reports indicated that immunization with M-44 had few adverse reactions and resulted in antibody production [39]. However, the persistence of organisms within organs in animal tests suggested that reactivation of infection could occur [40]. Interestingly, a similar live Ph 2 vaccine using the Nine Mile strain of *C. burnetii* was also tested in the USA, confirming Ph 2 antibody generation. Again, however, serious adverse reactions were encountered, further raising questions about the safety of these vaccines [40].

Various antigen extraction techniques were investigated in the following years. A chloroform methanol residue (CMR) vaccine from Ph 1 Henzerling strain *C. burnetii* was developed in the USA [41] and a trichloroacetic acid (TCA) extraction chemovaccine from purified Ph 1 *C. burnetii* was developed in former Czechoslovakia [42] [Table 1]. Both formulations were reported to retain the major immunogenic components of *C. burnetii* and hence strong immunogenicity, albeit less than whole-cell vaccines [41,42]. Minimal adverse reactions were observed in the clinical trials for each vaccine; however, the TCA chemovaccine was not recommended for persons with positive skin tests and/or serology test due to safety concerns [42]. Neither of these vaccines are currently available for public use; however, they remain of interest [43,44].

### Q-VAX

Currently, the only commercially available vaccine for human use against *C. burnetii* is Q-VAX, which has been licenced in Australia since 1989. Q-VAX is a purified formalin killed whole-cell *C. burnetii* Ph 1 vaccine developed from the Henzerling strain [45]. The efficacy of Q-VAX has been reported to be between 82 and 100% with long-lasting protection of up to 5 years. Trials for Q-VAX were conducted in the period from 1981–88, mainly in abattoir workers. Several common reactions were described in the cohort, including tenderness and erythema at the injection site and transient headache, all of which were self-resolving [46]. Some more serious and persistent reactions described included long-lasting lumps and an abscess at the injection site [46]. As a whole-cell killed vaccine, the risk of serious adverse reactions in those previously exposed to the bacterium, either by natural infection or by vaccination, necessitates prescreening and the vaccine is never given as a booster.

In Australia, the cost of production and supply of Q-VAX is heavily subsidized by the Australian government. In addition, medical professionals need to be trained in the administration and interpretation of the prescreening tests. The burden of at least two medical appointments for prescreening and vaccination, and the cost of the vaccine itself falls on either an employer or the individual seeking vaccination. Vaccination with Q-VAX is recommended

**Table 1. Experimental studies on potential Q fever vaccine candidates.**

| Vaccine type (mode of delivery) | Tested on | Effectiveness | | Adverse effects | | | Refs. |
|---|---|---|---|---|---|---|---|
| | | Lymphocyte Stimulation | Antibody Response | Injection site Reaction | Fever | Headache | |
| Ph 1 Henzerling strain (S) | H[†] | 66–95%+, variable | 80–100% | ✓ | | ✓ | [46,73–75] |
| CMR Ph 1 Henzerling strain (S, P) | H, G[‡], M[‡], Pr[‡] | 33–40% proliferative responses to antigens | 50–90% IgM<br>20–40% IgG | ✓ | ✓ | | [41,76–78] |
| Formalin inactivated Ph 1 and Ph 2 Nine Mile strain | M[‡] | NA | Significant IgG response | | | | [29] |
| Soluble bacterial extract Ph 1 and Ph 2 Nine Mile strain (SC) | M[‡], G[‡], Pr[‡] | NA | 100% +IgM<br>100% +IgG | | | | [79] |
| Ph 1, Ph 2 Nine Mile, Priscilla strain (IM) | G[‡] | NA | 100% high titres | | | | [14] |
| Recombinant protein subunit vaccine, purified proteins Ph 1 Nine Mile strain with TLR triagonist adjuvants (IM), | G[‡] | NA | 100% IgG responses | ✓ | | | [63] |
| M44 strain (S, C, O) | H, G[‡] | NA | 79–87%+ | ✓ | ✓ | ✓ | [39,80,81] |
| Ph 1 Xinqiao strain pooled antigens: com1, groEL, mip, ompA, ompH, p1, ybgF (S) | M[‡] | CD4+ proliferation responses to pooled antigens | | | | | [53] |
| Ph 1 TCA (unknown strain) | H | NA | 55% | ✓ | ✓ | ✓ | [42] |
| CMR Ph 1 Ohio strain (P) | M | NA | Significant IgG response | | | | [82] |
| Mimetic peptide vaccine m1E41920. | M[§] | NA | Significant IgG response | | | | [65] |
| Adenovirus vector encoding 14 *Coxiella burnetii* surface antigens (IM) | M[‡] | Increase in IFN-γ producing cells | | | | | [83] |
| Viral vector vaccine using subsets of 27 epitopes (IM) | M[‡], G, Pr | Antigen-specific IFN-γ and CD4+, CD8+ responses | | ✓ | | | [84] |
| OSP extracted Ph 1 Nine Mile strain, conjugated to tetanus toxoid (IM, S) | G[‡] | NA | No IgG response | | | | [64] |
| Ph 2 bacterin vaccine (S) | Sh[‡] | NA | Antibody responses | | ✓ | | [85] |

[†]Vaccinees were monitored for Q fever for 15 months to 7 years after vaccination.
[‡]Infection challenge performed in animals to test vaccine protection by assessing splenomegaly, bacterial loads in spleen and lungs, fever and weight loss [14,29,53,63,64,76,79–81,83–85].
[§]Immune sera from previously vaccinated and challenged mice reduced infections in naive mice [65].
C: Cutaneous; CMR: Chloroform:methanol residue; G: Guinea pig; H: Human; I: Intratracheal; IM: Intramuscular; M: Mouse; NA: Not assessed; O: Oral; OSP: O-specific polysaccharide; P: Peritoneal; Pr: Primate; Sh: Sheep; TCA: Trichloroacetic acid.

for persons over 15 years of age who may be at risk for *C. burnetii* exposure, including anyone who comes into close contact with animals, such as abattoir workers, farmers and veterinarians [47]. During prescreening, a detailed history is taken to identify exposure risks, previously diagnosed Q fever or vaccination. This is followed by a skin test and serological testing. The Q VAX skin test consists of an intradermal injection of a diluted 0.1-ml dose of 16.7 ng of killed *C. burnetii* bacteria and evaluates cell-mediated immune responses [45]. Interpretation of this test occurs 7 days after the injection and is considered positive if there is induration at the site of injection [47]. Humoral immune response is ascertained by detection of *C. burnetii*-specific antibodies [47]. Positive results in either of these tests indicate the possibility of previous exposure to *C. burnetii* and precludes the individual from vaccination [47]. Adherence to these safety protocols, risk of adverse outcomes and the costs associated with administration and production have prevented the widespread use of Q-VAX to prevent Q fever. Furthermore, implementation of this process has been described as challenging in large-scale vaccination programs [48]. However, more robust and cost-effective prescreening tests and the development of new generation vaccines that are less reactogenic could enable the wider spread use of Q fever vaccines.

## New vaccine development

Efforts to develop new generation vaccines have shifted the focus of investigations toward identification of the immunogenic components of *C. burnetii* to generate an adaptive immune response whilst avoiding adverse re-

sponses caused by unnecessary but highly reactogenic components of the bacteria. Studies have identified several immunogenic antigens of *C. burnetii* [49–56]. Interestingly, the location and function of these antigens are varied. Gerlach *et al.* [57] found that the majority of the known immunogenic proteins described were cytoplasmic, followed by those associated with the inner and outer membranes, and predominantly involved in functions relating to metabolism, gene expression, protein synthesis and replication. A number of these antigens have been tested in animal models for their protective capabilities (Table 1).

Current vaccine strategies are aimed at increasing immunogenicity of putative Q fever vaccine candidates and decreasing reactogenicity and adverse responses observed more frequently among those who have had prior exposure [58,59]. An improved Q fever vaccine should ideally provide protection at a comparable or higher level to the Ph1 whole-cell vaccine (Q-VAX), administered via a single dose regimen and be free from reactogenicity and post-vaccination hypersensitivity. The development of cell free media has enabled to simplify culture of *C. burnetii* and improve the development of whole-cell based preparations with reduced potential of post vaccination reactogenicity. Current vaccine development research has used immunogenic antigens [60], subunit vaccines (in the form of purified or recombinant peptides or proteins) and different LPS structure-based strategies [14,61], which have resulted in varying degrees of success in animal experimentation.

A disadvantage of a subunit vaccine is that the immunogenic response is not as robust as a whole-cell vaccine and therefore may be less effective in delivering a protective response. Various methods have been explored to promote better protective humoral and cellular immune responses. Adjuvants, such as Toll-like receptor (TLR) agonists, have been used to enhance innate immune receptor recognition of the peptide/epitopes so that a stronger immune response can be generated [62,63]. Purified Ph 1 O antigen polysaccharide has been conjugated to tetanus toxoid [64]. Vaccine delivery techniques have been investigated, including intratracheal delivered vaccines and the use of bacterial vectors [44,55].

Immunoinformatic technology has become an important tool for improved vaccine design. Peng *et al.* [65] used a phage library to develop a synthetic LPS-targeted peptide mimic vaccine to mimic a protective epitope of Ph 1 LPS. This vaccine was able to generate protective antibody responses in mouse models. Other researchers have used *in silico* analysis to identify candidate antigens involved in the generation of immune responses. Because the control of *C. burnetii* infection is dependent on T-cell mediated immunity, researchers have targeted the selection of epitopes predicted to have high affinity binding to MHC class I and MCH class II molecules to ensure CD8+ and CD4+ stimulation [66]. Vaccines are then designed and tested using these epitopes. Several promising candidates have been assessed, including a multi-epitope DNA vaccine tested in a mouse model [67]. This remains an active area of research [52,54].

## Conclusion

Q fever is a significant zoonosis that impacts a variety of people in addition to the traditional occupational risk groups of abattoir workers and animal handlers. The current Q fever vaccine, Q-VAX, although effective, is problematic in that it requires preassessment with serology and a skin test. In addition, there is a risk of an adverse reaction if given inadvertently to someone with prior exposure to the organism. An understanding of the immunological responses that could lead to chronic Q fever and endocarditis is an important first step in the development of a new vaccine to this disease. The expansion of regulatory T cells may be critical for the chronic evolution of Q fever and subsequent endocarditis. A specific cytokine response with TNF-α and IL-10 may help in identifying those at risk of endocarditis. There are currently a number of candidate antigens being assessed. These include subunit vaccines, multi-epitope DNA vaccines and synthetic LPS-targeted peptide mimic antigens. The continuing challenge is for the development of a simpler, safer and equally effective vaccine for Q fever.

## Future perspective

Protection against Q fever is most effectively achieved by vaccination. The ideal vaccine should be efficacious, safe to administer and cost-effective. The only licensed Q fever vaccine currently available for human use, Q-VAX, has demonstrated efficacy. Early studies, mainly in male abattoir workers, found that Q VAX was highly effective in preventing disease and provided long-lasting immunity [46]. Few Q fever cases in these high-risk occupations have been reported after vaccination [68]. Furthermore, a national Q fever vaccination program in Australia was able to reduce notification rates by approximately 50% [69]. However, it is not clear whether this high level of protection translates to populations with less risk of exposure. Recently, Q-VAX vaccination of a large group of

elderly individuals in The Netherlands reported that only 60% of vaccinees sustained seropositivity or demonstrated IFN-γ production (indicating a cell-mediated response) after 12 months [70].

As a killed whole-cell vaccine, Q-VAX is considered safe in Australia despite the risk of adverse reactions [16]. Prescreening requirements before vaccination are costly and time-consuming. Development of alternative prescreening assays could make the vaccination process more efficient [71,72]. Conversely, development of new vaccines that generate protective immune responses but are less reactogenic could eliminate the need for prescreening.

Advances in vaccine design have enabled new vaccines to be targeted to produce robust cell-mediated and humoral responses. Significant progress has been made in identifying and understanding *C. burnetii* immunogenic antigens and testing the protective capabilities in animal models. IFN-γ production could potentially be used as a correlate to accelerate identification of immunogenic antigens and also assist in determining efficacy of vaccine candidates [53,70] (Figure 3). Further studies into how these immunogenic antigens contribute to immunity in both naturally infected and vaccinated individuals would be beneficial. Furthermore, targeted vaccine development using immunoinformatic tools is a major step in vaccine design that can streamline the selection of epitopes for potential vaccine candidates that are efficacious and safe to use. This progress toward the development of a new Q fever vaccine provides confidence that a safe and effective vaccine will be available in the near future.

## Executive summary

**Background**

- Q fever is caused by the bacterium *Coxiella burnetii*.
- It is a zoonosis and, although previously thought primarily to affect workers in the cattle industries, it is now recognized to affect a wider range of people with nonoccupational exposure.

**The bacterium**

- *C. burnetii* is an obligate intracellular Gram-negative bacterium.
- The small cell variant can survive harsh external environmental conditions, whereas the large cell variant replicates and is metabolically active.
- *C. burnetii* undergoes antigenic phase variation.

**Clinical presentation of Q fever**

- The course of infection with *C. burnetii* varies among individuals.
- A small number of patients can develop endocarditis with subsequent significant outcomes.
- The current vaccine against Q fever (Q-VAX) is effective and has resulted in a reduction of cases in abattoir workers in Australia.
- The vaccine can cause significant adverse effects in those with prior exposure to the organism.
- As a consequence, both serology and a skin test need to be obtained before vaccination to prove no prior exposure.
- This prescreening increases both the cost and ease of delivery of the vaccine.

**Immune responses in Q fever**

- An understanding of the immunological mechanisms leading to protection is essential before the development of new vaccine candidates.
- IFN-γ plays an important role against *C. burnetii* by upregulating genes that contribute toward restricting intracellular replication of the bacterium.
- The expansion of regulatory T cells and specific cytokine responses with TNF-α and IL-10 may be implicated in the development of endocarditis.

**Vaccination against *Coxiella burnetii***

**Historical vaccine development**

- In 1948, a vaccine was developed from the Henzerling strain of *C. burnetii*, which was formalin killed and ether extracted. This proved to have significant adverse effects. Subsequent attempts to use both live attenuated and killed strains resulted in either unacceptable adverse effects of inadequate protection.

**Q-VAX**

- Q-VAX, has been licenced in Australia since 1989. It is a purified formalin killed whole-cell *C. burnetii* Ph 1 vaccine developed from the Henzerling strain. The efficacy of Q-VAX has been reported to be between 82 and 100% with long-lasting protection of up to 5 years.

**New vaccine development**

- Candidate antigens being assessed include subunit vaccines, multi-epitope DNA vaccines and synthetic LPS-targeted peptide mimic antigens.
- It is hoped that this ongoing work will result in the development of a simpler, safer and equally effective vaccine for Q fever.

### Author contributions

Writing – original draft: P Redden, R Norton, K Parker. Review and editing: S Henderson, P Fourie, L Agnew, J Stenos, S Graves, B Govan, N Ketheesan, R Norton.

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