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# Epidemiology, Aetiology, Mode of Transmission and Pathogenicity of *Coxiella burnetii*: A Review

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## Abstract

This review article describes the severity of Q fever zoonotic infection, including its epidemiological pattern and the reasons for its outbreaks. This paper highlighted the transmission routes of bacteria. It spreads through aerosol particles, mosquito bites, and exposure to domestic animals. Bacteria are also spread with animal products, such as unpasteurized milk. In India, cases of Q fever have been reported from Punjab, Uttar Pradesh, Delhi, Karnataka, Odisha, Pondicherry, and Tamil Nadu. *C. burnetii* exhibits a biphasic developmental cycle. This article explains the invasion of host cells by *C. burnetii* and its interaction with specific receptors, with different internalization pathways observed in phases I and II. It sketches out the interaction of *C. burnetii* with immune cells and their defense mechanisms. Continuous screening using modern diagnostic tools is crucial for identifying *C. burnetii* strains in endemic regions, thereby mitigating the pathogenesis and lethality caused by this pathogen. The present review also includes diagnostic methods used for identifying bacteria in blood and tissues.

## Keywords

Q Fever, Febrile Illness, *C. burnetii*, *Brucella*, Diagnosis, AES

## 1. Introduction

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*, a species belonging to the family Coxiellaceae and the order Legionellales. This bacterium infects them, causing severe pathogenesis and mortality. For the first time, this was reported in Queensland, Australia, in 1935 by the Health Department in Brisbane. The disease begins as a febrile sickness with flu-like symptoms among [1]. The most frequent and severe chronic condition that prevails is endocarditis, which presents a blood-culture negative endocarditis [2]. It was reported in immunocompromised

patients [2]. *Coxiella burnetii*, a strictly intracellular bacterium, harbors inside blood, liver, spleen, and lungs during the early stages of the disease. It occurs almost exclusively in patients who have pre-existing valvular disease or who are immunocompromised [3]. *Coxiella burnetii* is reported in biological samples, such as milk, and is highly concentrated in placental tissues and birth products of infected animals. The bacterium can become chronically shed in urine and faeces over time. It is well known that livestock is a significant source of human illness.

This pathogen has a broad host range, affecting a variety of animals, including agricultural animals, pets, wild mammals, reptiles, birds, and ticks. This bacterium also infects cattle, goats, and sheep. Serological studies indicate that the prevalence of *C. burnetii* infection and Q fever varies significantly among both farm animals and humans [4]. This bacterium has potential hosts, including cattle, goats, and sheep. Cats and dogs are known to act as reservoirs of *C. burnetii*. Dogs can contract infections through various routes, including tick bites, the consumption of placentas or milk from infected ruminants, and exposure to contaminated aerosols. It produces a variety of clinical syndromes.

## Morphological Features

*C. burnetii* is an obligatory intracellular bacterium that exhibits a highly pleomorphic coccobacillus morphology, with dimensions ranging from 0.2 to 0.4 micrometres in width and 0.4 to 1 micrometres in length. Although *C. burnetii* is classified as a Gram-negative bacterium, it does not stain effectively with the standard Gram staining method because of its distinct cell wall structure. However, it can be reliably visualized using the Gimenez stain [5]. A notable feature of *C. burnetii* is its ability to undergo phase variation, transitioning between smooth and rough phases. This variation is primarily attributed to mutations in its lipopolysaccharide (LPS) components [6]. Phase I occurs naturally in infected animals, insects, and humans and is known for its high contagiousness. During this phase, *C. burnetii* produces smooth, full-length lipopolysaccharide (LPS) on its cell surface. The smooth LPS facilitates the bacterium's evasion of the host's immune system, contributing to its virulence and enabling it to establish and maintain persistent infections within the host. On the other hand, Phase II can only be produced *in vitro*, meaning it occurs in laboratory settings rather than naturally within the host. It is a result of inducing changes in the bacterium through multiple passages in cell cultures, embryonated egg cultures, or synthetic medium. Consequently, *C. burnetii* transforms non-virulent organisms during this process. In Phase II, the bacterium produces truncated rough LPS lacking the O-antigen region. The composition of Phase II LPS is distinct, including 2-keto-3-deoxyoctulonosic acid (KDO), D-mannose, D-glycero-D-manno-heptose, lipid A, or a lipid A analogue. Additionally, the fatty acid mixture in Phase II LPS is more complex than that in Phase I [7]. Indeed, Phase I LPS of *C. burnetii* consists of additional components such as L-virenose, dihydrohydroxystreptose, galactosamine, and uronyl-(1,6)-glucosamine, which are not present in Phase II LPS [6].

## 2. Epidemiology

### 2.1. Disease Transmission

Q fever is a worldwide zoonotic infection characterized by an epidemiological pattern consisting of sporadic cases, endemic situations, and outbreaks of unsuspected magnitude, as occurred in the Netherlands. Q fever is a zoonosis with a worldwide distribution, except in New Zealand [8]. Q fever has a global distribution, but its epidemiological characteristics differ by region, ranging from areas where it is endemic or hyperendemic to those experiencing large-scale outbreaks [9]. An outbreak of Q fever and its long-term effects have also been documented in the Netherlands, with more than 4,000 reported cases [10].

#### 2.1.1. Aerial Spread

*Coxiella burnetii* is primarily transmitted through airborne aerosols. It enters the human respiratory tract via inhalation of contaminated particles, such as infected fomites. This is considered the main route of human infection. Environmental factors, such as vegetation, soil moisture, and agricultural land, increase the risk of *C. burnetii* transmission [11]. Contamination with *Coxiella burnetii* aerosols can occur directly from the birthing fluids of infected animals, which may infect the newborn, placenta, or wool. Bacterium is highly resilient in the environment and can survive for several weeks in areas frequented by animals. It can also be dispersed by wind, allowing Q fever to develop in individuals without direct contact with animals. Additionally, *C. burnetii* can be transmitted through the consumption of unpasteurized milk and dairy products. Due to its high heat resistance, the bacterium is not easily destroyed by standard heating methods, making raw or unpasteurized dairy a significant source of human infection [12].

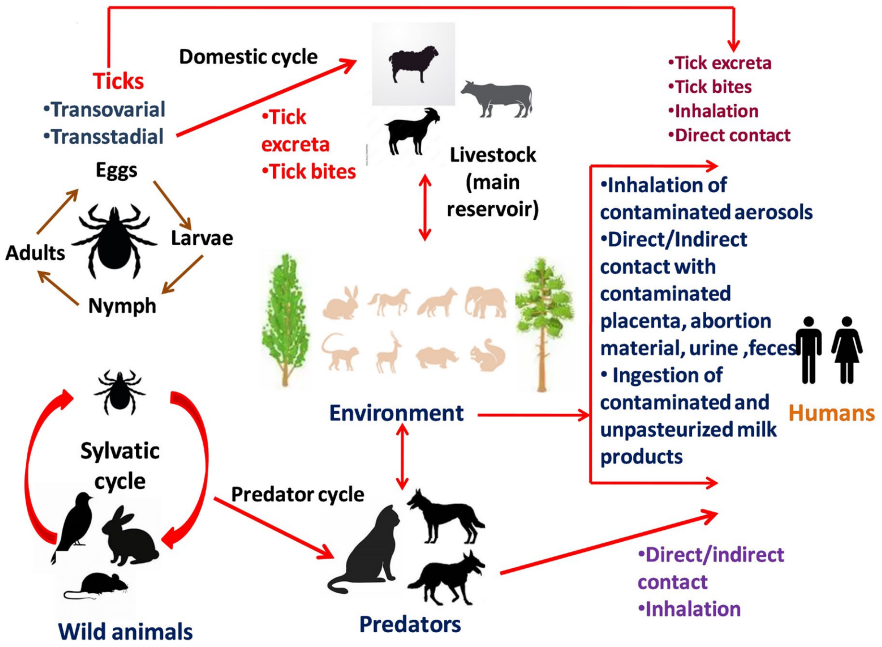



Figure 1. Transmission of bacteria from animals to animals and animals to humans.

Person-to-person transmission of *Coxiella burnetii* is considered extremely rare. However, isolated cases of human Q fever have been reported following contact with infected parturient women, for example, an obstetrician involved in performing an abortion. Other rare modes of transmission include transplacental transfer leading to congenital infections, exposure during autopsies, intradermal inoculation, and blood transfusion. Although *C. burnetii* has been isolated from arthropods—primarily ticks—tick-borne transmission to humans appears to be minimal. Nevertheless, two cases of coinfection with *Rickettsia conorii* and *C. burnetii* were reported in individuals residing near Montpellier, suspected to have resulted from tick bites. Such occurrences are infrequent, and current knowledge on this transmission route is limited. Therefore, the role of this pathway in the transmission of Q fever to humans and wild animals remains uncertain [8] (**Figure 1**).

#### 2.1.2. Transmission through Poultry Products

Q fever can be transmitted through poultry products, as *Coxiella burnetii*, the bacteria causing Q fever, can be found in various poultry and their products. While cattle, sheep, and goats are primary reservoirs, poultry, such as chickens, ducks, geese, and turkeys, can also carry and transmit the bacteria. People can contract Q fever by inhaling contaminated dust, consuming unpasteurized milk or dairy products.

For the detection of *Coxiella burnetii* in hens, turkeys, ducks, geese, and pigeons, the microagglutination (MA) test is used to determine seropositivity in blood samples. This analysis is performed using the highest seropositivity rate, which was noted in farm hens due to natural infection from proximity to humans in endemic areas. The lowest seropositivity rates are prevalent in areas away from farmland. A similar higher prevalence of infection was observed in birds inhabiting or feeding near infected livestock, which shows the presence of secondary or incidental hosts rather than primary reservoirs in the *C. burnetii* transmission cycle. The presence of *C. burnetii* was tested in blood samples of domestic quail (*Coturnix coturnix japonica*) and domestic Muscovy ducks (*Cairina moschata*) (3% each), domestic chickens and mallards (*Anas platyrhynchos domesticus*) (2% each), carrion crows (*Corvus corone*) (37%), jungle crows (35%), and wild rock doves (*Columba livia*) (6%) [13]. Seropositivity was also tested in rodents and chickens from poultry farms. The bacterium was detected in 16.2% of *R. rattus*, 8.8% of *R. tanezumi*, and 25% of chicken samples. It confirms transmission of *Coxiella burnetii* from infected birds to humans and between other animal hosts [14]. Seropositivity was found to be notably higher in farmyard animals. Buffaloes showed higher transmission risks due to their enhanced seropositivity. The overall seroprevalence rate was 11.7%. The presence of bacteria in livestock indicates a pathogenic association between human cases and exposure to infected ruminants [15].

### 2.2. Major Risks

Q fever primarily affects individuals with occupational exposure to animals, including livestock farmers, slaughterhouse workers, veterinarians, and researchers

working with animals or in laboratories. It is especially common among those working in cattle yards. In addition to cattle, other hosts of *Coxiella burnetii* include horses, pigs, dogs, cats, camels, buffaloes, and a variety of wild and domestic birds such as chickens, pigeons, ducks, geese, and turkeys. The illness often begins with mild fever but can progress to a more severe condition marked by pronounced clinical symptoms. Humans typically become infected by inhaling dust contaminated with the bacterium shed by subclinically infected animals [16]. Extensive bacterial proliferation within the lungs can lead to acute Q fever pneumonia, which is more commonly observed in individuals with compromised immune systems. *Coxiella burnetii*—an intriguing organism—was initially classified as *Rickettsia burnetii* due to its similarities with the Rickettsia genus. However, subsequent phylogenetic studies, particularly those based on 16S rRNA sequencing, prompted its reclassification. Globally, the incidence of *C. burnetii* infection is showing a rising trend, particularly among high-risk occupational groups [17].

### 2.3. Geographical Patterns and Seasonal Variations

The occurrence of Q fever, or Coxiellosis—a zoonotic disease—varies widely across different geographic regions. In Europe, a distinct seasonal trend is observed, with a higher number of acute Q fever cases reported during the spring and early summer months [6]. In some regions, a higher incidence of Q fever is observed during the winter months, while in tropical areas, its occurrence often coincides with the rainy season. For instance, in Queensland, Australia, the disease typically appears in May following peak rainfall in February. Q fever is a notifiable disease and is recognized as an infection capable of affecting a wide range of animal species. In India, the first recorded outbreak of human Q fever occurred in Dehradun in 1954. As the world's largest producer of milk, India has a vast dairy livestock population, and dairy farming remains a vital source of income in rural communities. Consequently, Q fever infections in domestic animals have been widely reported across the country, with significant cases documented in Punjab, Uttar Pradesh, Delhi, Karnataka, Odisha, Puducherry, and Tamil Nadu [12].

### 2.4. Life Cycle

*C. burnetii* exhibits a biphasic developmental cycle (**Figure 2**). This bacterium undergoes a complex life cycle, first proposed through observations using an electron microscope, which involves a non-replicating minor cell variant (SCV) and a replicating large cell variant (LCV) [18].

The SCV is a compact, dense, and dormant form of *C. burnetii* that is highly resistant to environmental stresses. SCV are coccoid in shape, characterized by their small size, with dimensions ranging from 0.2 to 0.5 μm. They display a highly dense structure, packed with a thick peptidoglycan layer and possess a reduced periplasmic space. Minor Cell Variants (SCV) of *C. burnetii* are metabolically inactive and highly infectious forms. They are more stable under environmental

conditions than LCV and exhibit remarkable resistance to physical and chemical stresses.

On the other hand, the Large Cell Variants (LCV) of *C. burnetii* are larger (>0.5 μm), dense, and metabolically active, and are responsible for replication within host cells [5]. LCV is characterized by a thin layer of peptidoglycan and a larger periplasmic space compared to SCV [15]. *C. burnetii* possesses an active Type 4B Secretion System (T4BSS), which plays a crucial role in forming the bacterium's intracellular niche [6]. The minor cell variant (SCV) of *Coxiella burnetii* can remain viable for 7 to 10 months on wool at room temperature, over a month on fresh meat, and for more than 40 months in milk. While SCVs can be inactivated by 2% formaldehyde, they have still been recovered from tissues preserved in formaldehyde for 4 to 5 months [5].

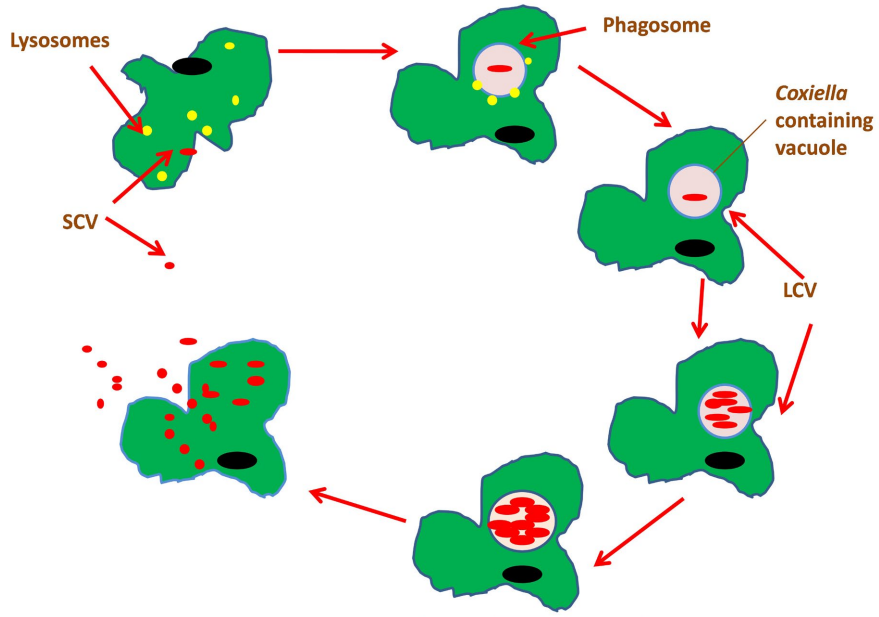



**Figure 2.** Biphasic developmental cycle: *C. burnetii*.

## 2.5. Phase Variation of *C. burnetii*

Phase I strains of *Coxiella burnetii*, such as Nine Mile I (NMI), are virulent and capable of causing disease in animal models. Phase I represent the natural phase occurring in infected animals, insects, and humans. It is highly contagious and produces smooth, full-length LPS. This phase contributes to *C. burnetii*'s ability to evade the host's immune system and establish persistent infections. Phase II can only be obtained *in vitro* through the conversion of phase I to avirulent organisms after serial passages in cell cultures, embryonated egg cultures, or synthetic medium. In this phase, *C. burnetii* produces truncated rough LPS lacking the O-antigen.

In contrast, Phase II strains, like Nine Mile II (NMII), are non-virulent. The antigenic variations observed in *C. burnetii* resemble those found in members of the Enterobacteriaceae family. This bacterium undergoes a unique process of

phase variation, characterized by two distinct phases of change from smooth to rough. The term "phase-variation" was initially used to describe the serological behaviour of *C. burnetii* and the mutational variations in the lipopolysaccharide (LPS) (**Figure 2**). Despite the absence of morphological variation between the two phases, the altered LPS composition significantly impacts the bacterium's virulence, with phase II being less pathogenic. However, morphological variation is absent between the two phases. But LPS is the sole factor responsible for the virulence of the two phases [19] (**Figure 2**).

### 2.6. Route of Infection

Phagocytic cells, specifically monocytes and macrophages, are the primary target cells for *C. burnetii* in humans and animals. In humans, most infections with *C. burnetii* occur following the inhalation of infected aerosols [5]. When *Coxiella burnetii* enters the respiratory tract, alveolar macrophages in the lungs are the first cells to be infected. *In vitro*, the bacterium can be cultured using several cell lines, including Vero cells, fibroblasts, and murine macrophages. For in vivo propagation, embryonated chicken eggs and laboratory animals such as mice and guinea pigs are commonly used [6].

### 2.7. Genomic Variations

The pleomorphic, obligately intracellular, Gram-negative, rod-shaped bacteria are called *Coxiella burnetii*. It displays antigenic shift and phase fluctuation. When sub-cultured in cells or embryonated eggs, it can take on a noninfectious phase II form, while its highly contagious phase I form is observed in animals [19]. A tick was the source of the *C. burnetii* RSA493 strain [5]. With a facultative plasmid and a linear chromosome, it has a distinct genetic composition. Although all strains of *C. burnetii* share a linear chromosome, one distinctive genetic trait is the integration of nucleotide sequences, which are approximately 16 kilobases long [16]. With genome sizes ranging from 1.9 to 2 megabase pairs (Mbp) between strains, *C. burnetii* demonstrates significant genomic variability. Bacteria may include any one of the five plasmid types listed below [20] (**Table 1**).

Using restriction endonuclease digestion and sequence-based typing techniques, the genomic diversity of *Coxiella burnetii* strains has been classified into several genomic groupings linked to pathogenesis.

Group I to III: These strains, which have been recovered from human blood, ticks, chiggers, cow's milk, and goat miscarriages, are frequently associated with acute Q fever and carry the QpH1 plasmid (**Table 1**).

Group IV: Consists of the QpRS plasmid and is linked to endocarditis and other chronic, focused infections; it is frequently identified from heart valves and abortion supplies (**Table 1**).

Group V: Does not contain plasmids; however, the chromosome does contain plasmid-like sequences. These strains have also been linked to hepatitis and endocarditis [18] (**Table 1**).

Table 1. *Coxiella burnetii* strains and their origin.

| Genomic Group | Strain | Plasmid Type | Origin |
|---|---|---|---|
| I | Nine Mile I RSA493 | QpH1 | Tick, Montana |
| I | Nine Mile Crazy RSA514 | QpH1 | Placental tissue (guinea pig, 343 days) |
| I | Nine Mile II RSA439 | QpH1 | Tick, Montana → Long-term *in vitro* |
| I | Ohio RSA270 | QpH1 | Cow's milk, Ohio |
| II | Henzerling RSA343 | QpH1 | Human blood, Italy |
| II | Henzerling RSA331 | QpH1 | Human blood, Italy |
| III | Idaho Goat Q195 | QpH1 | Goat placenta, Idaho |
| IV | PQ238 | QpRS | Human heart valve, California |
| IV | MSU Goat Q177 | QpRS | Goat cotyledon, Montana |
| V | G Q212 | Integrated plasmid sequence | Human heart valve, Nova Scotia |
| V | S Q217 | Integrated plasmid sequence | Human liver biopsy, Montana |
| VI | Dugway 7D 77-80 | QpDG | Rodents, Utah |
| VI | Dugway 7E 65-68 | QpDG | Rodents, Utah |

The severity of Q fever is also influenced by the climatic factors and genomic diversity of *Coxiella burnetii*, with distinct genomic groups exhibiting variable virulence profiles. The pathogenic potential of various *Coxiella burnetii* strains is closely associated with the presence of specific plasmids. These plasmids display a high level of sequence homology, suggesting their conserved nature across strains. They are thought to carry key virulence genes that significantly influence the level of virulence exhibited by different *C. burnetii* isolates [19].

Among all the strains, Group I shows high infectivity, which is due to the presence of QpH1 plasmids. Group I strains were found associated with the most severe disease, while strains from groups II-V demonstrated moderate to low virulence. The genomic groups I to VI also exhibited high virulence, febrile response, splenomegaly, and serological reactivity. Notably, a sustained febrile reaction was found in over 50% of the animals within each group. The intensity of splenomegaly and antibody responses at 14 days post-infection reflects the severity of fever caused by strain-specific virulence. The difference in infectivity among different strains of *Coxiella burnetii* was due to a potential difference in gene content. These genes also serve as molecular determinants of virulence, which can aid in assessing risk, strain identification, diagnostics, and vaccine development strategies [21]. In animals tested positive for virulent group I strains, a marked reduction in potential $CD4^+$ T cell populations were observed; clinically, these effects are considered adverse immunomodulatory effects. Group VI strain-infected animals elicited no significant clinical signs.

## 3. Pathogenicity and Host Interaction

### 3.1. Infection and Its Persistence

Phagocytic cells, specifically monocytes and macrophages, are the primary target cells for *C. burnetii* in both humans and animals. In humans, the majority of infections with *C. burnetii* occur following the inhalation of infected aerosols [5]. Upon inhalation, *Coxiella burnetii* primarily targets alveolar macrophages in the lungs as the initial site of infection. *In vitro* cultivation of the bacterium is possible using various cell lines, including Vero cells, fibroblasts, and murine macrophages. For *in vivo* studies, *C. burnetii* is commonly propagated in embryonated hen eggs and laboratory animals such as mice and guinea pigs [6].

### 3.2. Internalization of *C. burnetii*, Target Cells and Receptors

In vertebrates, *Coxiella burnetii* primarily infects phagocytic cells such as monocytes and macrophages, utilizing them as its primary host cells. Interestingly, similar to *Legionella* and *Francisella* species, *C. burnetii* also possesses the ability to survive within the amoebae. The bacterium's entry into host cells is mediated by specific receptors, with distinct internalization mechanisms observed between Phase I and Phase II forms. During Phase I infection, *C. burnetii* engages Toll-like receptor 4 (TLR4), *avβ*3 integrin, and integrin-associated protein (IAP), enabling its uptake by phagocytic cells. A unique strategy involves the bacterium targeting and depleting CR3 receptors on the surface of these cells, leading to the development of membrane ruffles or pseudopodia. These structures facilitate the bacterium's internalization. Furthermore, TLR4 activation by *C. burnetii* triggers tyrosine kinase signaling and cytoskeletal rearrangement, promoting bacterial survival and establishment within the host cell. In contrast, during Phase II, *C. burnetii* utilises a different receptor profile for entry, primarily interacting with *avβ3* integrin and IAP, thereby bypassing the CR3-mediated pathway [19].

*C. burnetii*'s sophisticated intracellular survival mechanisms and immune evasion strategies add considerable complexity to disease management [22]. In vertebrates, *C. burnetii* primarily targets phagocytic cells such as monocytes and macrophages (**Figure 3**). Interestingly, like Legionella and Francisella species, *C. burnetii* survives within amoebae. The invasion of host cells by *C. burnetii* involves specific receptors, with different internalisation pathways observed in phases I and II. During Phase I, *C. burnetii* interacts with toll-like receptor 4 (TLR4), v3 integrin, and integrin-associated protein, facilitating its internalization into host cells [6]. *Coxiella burnetii* employs an advanced strategy to engage host cell receptors during its invasion of phagocytic cells. In Phase I, the bacterium selectively downregulates CR3 receptors on the surface of these immune cells, triggering the formation of membrane ruffles, also known as pseudopods. These structures aid in the bacterium's uptake by the host cell. Furthermore, its interaction with Toll-like receptor 4 (TLR4) activates tyrosine kinase signalling pathways and induces cytoskeletal rearrangements, creating a favourable intracellular environment for bacterial survival and replication. In contrast, during Phase II, *C. burnetii* utilizes

an alternative entry mechanism. Instead of targeting CR3 receptors, it engages *avβ3* integrin and integrin-associated protein (IAP) to facilitate its internalization into macrophages [19].

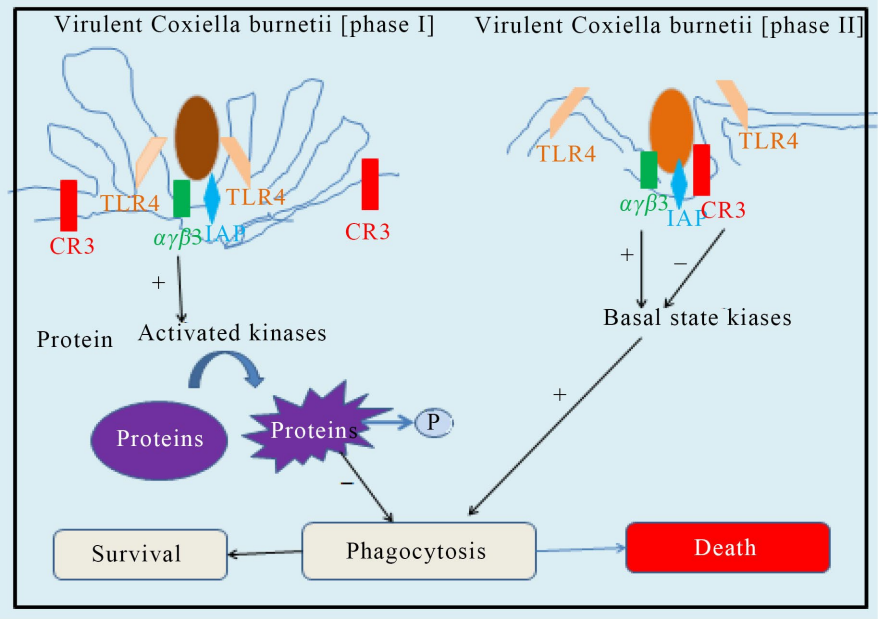



**Figure 3**. Phagocytosis of the bacteria by the macrophage cells.

### 3.3. Symptoms and Clinical Issues

Q fever most frequently presents as a flu-like illness, marked by symptoms including high fever, chills, headache, muscle pain, and general fatigue. In more severe cases, patients may develop varying degrees of pneumonia and hepatitis, which can lead to complications, while acute Q fever is generally self-limiting and resolves on its own. Endocarditis is one of the most significant chronic presentations of Q-fever [23]. Approximately 60% of Q fever cases are asymptomatic, meaning infected individuals show no apparent symptoms of the disease. Among the remaining 40% of patients who do experience symptoms, the majority (38% of the 40%) will have a mild form of illness that does not require hospitalization. Hospitalized patients represent only a small proportion, approximately 2% of all infected individuals. The pathological makeup of Q fever varies considerably; in humans, illness can manifest as either an acute or chronic infection [6].

### 3.4. Acute Q Fever

Symptoms of acute Q fever are typically flu-like but can vary significantly. It might cause pneumonia, inflammation of the brain or its covering (encephalitis or meningitis) or inflammation of the liver (hepatitis). Symptoms might include Fever, Extreme fatigue, Chills, Sweats, Muscle aches, Sensitivity to light (photophobia), severe headaches, Nausea and vomiting, diarrhoea, Cough, Chest pain, Stomach pain, Rash-like spots under your skin (purpura), Shortness of breath (dyspnea) [24].

### 3.5. Chronic Q Fever (Persistent Q Fever)

Persistent Q fever is relatively uncommon, affecting only approximately 5% of infected individuals. Patients with chronic Q fever are at a significantly increased risk of developing endocarditis, a serious condition that involves inflammation and infection of the heart valves. Chronic Q fever starts months to years after your initial *C. burnetii* infection. While it most commonly affects the heart, heart valves and blood vessels, the symptoms can vary depending on which parts of the body are affected. Symptoms of chronic Q fever include Low-grade fever, Night sweats, Weight loss, Fatigue, Shortness of breath, and Swelling of your legs or feet [24] (**Figure 4**).

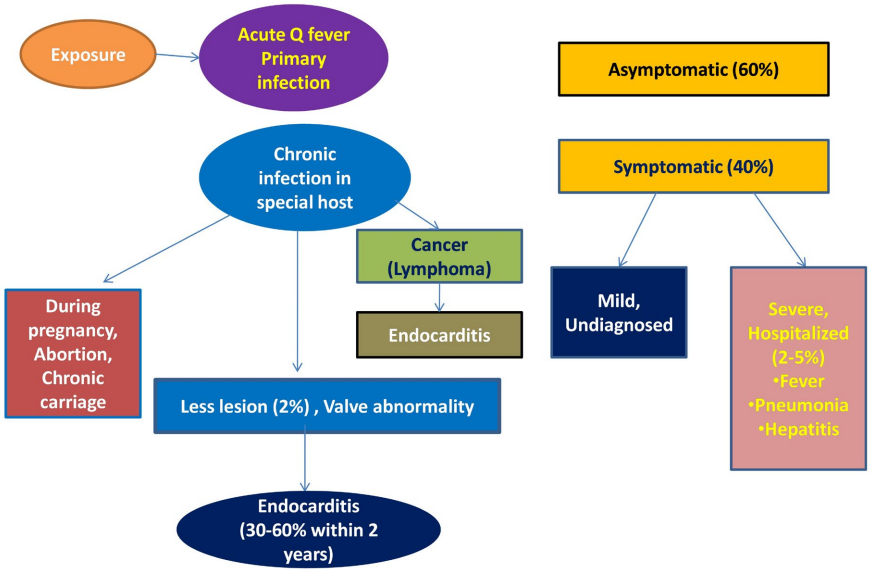



**Figure 4.** Sign and symptoms associated with acute and chronic Q-fever.

### 3.6. Host Immune Response

*Coxiella burnetii* is a facultative intracellular pathogen that replicates within the acidic environment of phagocytic cells, relying on cell-mediated immunity for adequate clearance and control. Its infection process involves the innate immune system, particularly monocytes, macrophages, and natural killer (NK) cells. Monocytes and macrophages serve as the primary host cells, exhibiting variable responses to invading pathogens. Upon interaction with monocytes, *C. burnetii* induces cellular polarization and triggers the release of cytokines.

Nitric oxide synthase (NOS) catalyses the conversion of L-arginine to citrulline, generating reactive nitrogen species (RNS) that play a crucial role in controlling infections [25]. During the acute phase of infection, circulating monocytes predominantly polarise toward the pro-inflammatory M1 phenotype, which is characterized by the production of cytokines such as IFN-$\gamma$, IL-6, and IL-12, as well as the expression of the chemokine receptor CCR7 [26].

Tissue-resident macrophages tend to adopt an atypical M2 phenotype during infection, marked by the expression of CXCL8 and specific cytokines. This M2

phenotype, which functions in contrast to the pro-inflammatory M1 type, supports the survival and proliferation of *Coxiella burnetii*. In chronic infections, macrophages are reprogrammed to adopt an M2 profile, resulting in the secretion of immunosuppressive cytokines, including IL-10 and TGF-β, which facilitates the pathogen's persistence and spread. M1 and M2 macrophages each shape the adaptive immune response by guiding T cells to differentiate into the TH1 or TH2 subsets, respectively [27].

Cytokines IL-4 and IL-10, produced by M2 macrophages, promote a TH2-type immune response. In contrast, cytokines such as IFN-$\gamma$ and IL-12, secreted by M1 macrophages, stimulate a TH1 response, thereby enhancing macrophage activity through a positive feedback mechanism. Moreover, macrophages from guinea pigs immunized with phase I *C. burnetii* can degrade the pathogen independently of antibodies, suggesting T cell-dependent activation and concurrent antigen presentation for T cell priming. Pro-inflammatory cytokines, including TNF-$\alpha$ and IL-12, play a crucial role following *C. burnetii* infection [28]. These are directly involved in immunity and the control of infectious diseases. IL-12 plays a pivotal role in promoting the differentiation of T cells into the Th1 subset and acts in synergy with TNF-$\alpha$ to amplify IFN-$\gamma$-dependent elimination of *C. burnetii* [29].

- **Neutrophils**

Neutrophils serve as the initial responders to pathogenic infections, capable of recognizing, engulfing, and destroying invading microbes. Both Phase I and Phase II strains of *Coxiella burnetii* are capable of infecting neutrophils, although the infection occurs at comparatively low frequencies. Infected neutrophils can further facilitate the spread of the pathogen by transmitting it to macrophages during the process of apoptotic cell clearance. In SCID mice, a delayed neutrophil recruitment to the infection site has been observed, which is associated with reduced levels of pro-inflammatory cytokines—an effect linked to the immune evasion strategies of *C. burnetii* [30]. Consequently, while neutrophils contribute to reducing clinical symptoms through vaccine-induced responses, they do not significantly aid in clearing the bacterial infection [31].

- **Dendritic cells**

Dendritic cells (DCs) are essential immune surveillance cells that continuously scan the body for invading pathogens. Functioning as sentinels, they detect and respond to foreign antigens. As specialized antigen-presenting cells (APCs), DCs play a pivotal role in initiating and regulating adaptive immunity by activating T cells. They capture antigens in peripheral tissues, process them, and migrate to lymph nodes, where they present antigen-peptide complexes via MHC molecules to T cells, thereby triggering a targeted immune response [32]. This interaction is key in orchestrating cell-mediated immunity. Human DCs infected with phase I *Coxiella burnetii* exhibit reduced expression of maturation markers, including MHC class II, CD80, CD86, CD83, and CD40 [33]. In particular, partially matured DCs infected with *C. burnetii* exhibit downregulation of TLR4, TLR3, STAT1, and interferon response genes [29], thereby impairing their ability to initiate robust

immune responses. Despite this, DCs remain central to coordinating both innate and adaptive immunity. The chemokine receptor CCR7 facilitates their migration to lymph nodes, where they initiate T cell activation [33].

The importance of this mechanism was highlighted by the lack of protection in phase I-vaccinated mice that were deficient in MHC-II molecules when exposed to *C. burnetii* infection. Additionally, the robust activation of macrophages begins with the recognition of pathogenic elements by Toll-like receptors (TLRs), which triggers transcriptional pathways that release mediators to enhance adaptive immune responses. Research on mice lacking TLR2 has shown impaired production of pro-inflammatory cytokines, including TNF-$\alpha$ and IL-12, following *C. burnetii* infection. IL-12 facilitates T cell differentiation into TH1 cells and works synergistically with TNF-$\alpha$ to promote IFN-$\gamma$-mediated destruction of *C. burnetii*. Thus, the protective roles played by macrophages during *C. burnetii* infection and vaccination involve a collaborative effort between innate and cell-mediated immune responses. Neutrophils, as the initial responders to infections, possess the ability to identify, engulf, and eliminate pathogens without relying on adaptive immune mechanisms. Phase I and II strains of *C. burnetii* infect neutrophils at a diminished infection rate, and these infected neutrophils can further replicate and invade macrophages during the clearance of apoptotic neutrophils. An aerosolized infection model in SCID mice revealed a delayed influx of neutrophils at the site of infection, attributed to a decrease in pro-inflammatory cytokines due to *C. burnetii*'s immune evasion strategies. Mice depleted from neutrophils exhibited decreased body weight, increased splenomegaly, and heightened bacterial load after being infected with *C. burnetii*, indicating their role in mitigating clinical disease and facilitating bacterial clearance. In contrast, phase I vaccinated and neutrophil-depleted mice showed reduced body weight and splenomegaly, with no significant difference in bacterial clearance when compared to control mice.

This implies that neutrophils contribute to the vaccine-induced alleviation of clinical symptoms rather than directly aiding in bacterial clearance. Dendritic cells (DCs), also known as "alarm cells", act as immune sentinels that connect innate and adaptive immune responses. Their capacity to capture antigens and present them as peptide-MHC complexes to T cells is crucial for orchestrating cell-mediated immune responses to infections [34]. *In vitro* investigations involving *C. burnetii* have indicated that virulent phase I strains disrupt DC maturation. Human DCs infected with phase I displayed lower levels of maturation markers such as MHC class II, CD80, CD86, CD83, and CD40 compared to the heightened expression observed in phase II-infected DCs. This research also revealed that phase II-induced DC maturation occurs independently of TLR4, resulting in increased production of IL-12 and TNF-$\alpha$. In contrast, Grovel *et al.* documented partial maturation of *C. burnetii*-infected DCs with down-regulated expression of TLR4, TLR3, STAT1, and interferon response genes. This finding is further corroborated by a recent study, which shows that phase II-infected DCs exhibit reduced MHC expression and impaired maturation. However, the administration of IFN-$\gamma$ was

able to counteract the inhibitory effects of phase II on DCs. Many of these studies involved monocyte-derived dendritic cells, which have the limitation of inadequate IL-12p70 production, a heterodimer essential for the activation and differentiation of TH1 cells [35]. The conclusion of phase I WCV triggered cellular reactions in CCR7-deficient mice, underscoring the importance of dendritic cell (DC) migration to lymph nodes in initiating cell-mediated immune responses. In murine models, the injection of phase II WCV-activated bone marrow-derived dendritic cells (BMDCs) resulted in a reduction of bacterial load, showcasing the protective function of antigen-stimulated BMDCs.

This was linked to elevated proliferation of Th1 CD4+ helper T cells, an increased bias toward Th17 cell expansion, and a decline in regulatory T cell populations. T-bet, a transcription factor found in both lymphoid and myeloid cells, is necessary for DCs to produce IFN-$\gamma$, TNF-$\alpha$, and activate antigen-specific T cells. T-bet knockout (KO) mice showed notable body weight loss and splenomegaly when compared to phase I-vaccinated wild-type mice, highlighting the crucial role of DCs in the initiation of T cell-mediated responses [36].

**1) Cell-mediated responses**

These adaptive immune responses ultimately defeat the infections that reside inside cells. Since cell-based responses directly stimulate infected cells to kill the bacterium, they primarily aid in the removal and management of *C. burnetii*. The antibody response is unable to reach *C. burnetii* since it is a cell-resident organism. Therefore, one important strategy to prevent *C. burnetii* infections is antibody-mediated T cell activation, which easily eliminates infected cells [37]. T cells are crucial for initiating cellular defenses. This is evident from the fact that mice with a compromised immune system, known as SCID mice, are unable to eradicate *C. burnetii* strains NMI and NMII. The infection can be eliminated by giving these mice CD4+ and CD8+ T lymphocytes from infected animals. On the other hand, healthy mice devoid of T cells are more susceptible to infection. When SCID mice are given splenocytes and T cells from immunocompetent mice, they can prevent spleen expansion, lower splenic bacterial counts, and maintain their health following infection. When immune cells at the infection site absorb *C. burnetii*, the immunological response starts. The germs are transported from the lungs to adjacent lymph nodes by dendritic cells in the airways, where T cells can be activated via the lymphatic fluid. The germs can also reach the lymph nodes. Mature dendritic cells in the lymph nodes present fragments of the bacterium to T cells of the immune system, triggering a reaction. *Coxiella burnetii* induces apoptosis during the early stage of infection via a caspase-independent pathway in human monocytic THP-1 cells [38].

Following an infection, mice deficient in MHC class II molecules exhibited weight loss, suggesting an inability to mount an effective immune response. Additionally, mice lacking MHC II or those immunized with aluminium-adjuvanted vaccines derived from inactivated organisms fail to show a reduction in bacterial load post-vaccination. Upon recognizing bacterial antigens, T cells differentiate into effector

CD4+ T cells, particularly into the Th1 subtype, which produces key cytokines such as IL-2 and IFN-$\gamma$. These cytokines play a crucial role in supporting T cell functions and enhancing the immune response [39] [40]. Because *C. burnetii* only lives outside of cells for a brief period, antibodies are crucial during that time. Mice without B cells grow larger spleens and more bacteria, demonstrating that vaccine protection depends on B cells. IgM antibodies, which play a crucial role in preventing infection and do not require T cells, form the foundation of this immunity [39]. According to [40] individuals vaccinated with inactivated pathogens demonstrate enhanced protection. In general, robust immunity against *Coxiella burnetii* relies on the coordinated responses of both T cells and B cells.

## 4. Diagnosis

### 4.1. Histology and Histochemical Methods

A range of diagnostic methods is employed to detect Q fever. For the detection of morbidity related to Q fever endocarditis and vascular infection, no single method is sufficient to find 100% characterisation. Hence, serological, histopathological, immunological, and molecular diagnosis is performed for the presence of *C. burnetii* in hospital-collected samples. There is no single test with a 100% predictive value for Q fever endocarditis or vascular infection is available. Hence, both PCR and serological, histopathological, and molecular examinations are performed on the samples.

One key technique involves examining the Small Cell Variant (SCV) forms to identify the morphology of *Coxiella burnetii*. Classified as a Group B biological agent, *C. burnetii* poses a significant health risk to humans. Consequently, all laboratory procedures involving this pathogen must be performed in facilities equipped with appropriate biosafety protocols. Both the Large Cell Variant (LCV) and Small Cell Variant (SCV), including their spore-like forms, can be visualised under a microscope. For the culture of bacteria, serum samples of guinea pigs, mice, and embryonated eggs are used to maintain *in vitro* cell cultures. The Gimenez staining method (Gold standard method) is used to detect bacteria in infected cell cultures in laboratories. *C. burnetii* is characterised by a very significant number of thin, pink-stained coccobacilli against a blue or green background.

Two primary forms of *C. burnetii* strains—phase I (virulent) and phase II (avirulent)—have been distinguished using single-nucleotide polymorphism (SNP) genotyping. Analysis of these isolates revealed 20 genomic polymorphisms across 1 - 18 open reading frames (ORFs), many of which featured partial deletions, point mutations, or insertions. Both minor cell variants (SCVs) and significant cell variants (LCVs) represent infectious forms of *C. burnetii*. The SCV form is remarkably resilient, capable of surviving in the soil for extended periods without a host. It demonstrates strong resistance to osmotic, oxidative, and thermal stress, as well as desiccation, pressure, and ultraviolet radiation.

### 4.2. Serological Diagnosis

Blood serum and vaginal swab samples are analysed by ELISA and PCR (com1 and IS1111) [41]. Since most clinical samples are not collected within the first two weeks of symptom onset—when *C. burnetii* DNA may still be detectable—Q fever diagnosis primarily relies on serological testing. Several serodiagnostic methods, including indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and complement fixation assay (CFA), are effective for identifying acute Q fever, offering comparable levels of sensitivity and specificity. These assays are capable of distinguishing between IgM and IgG antibodies and can differentiate between the Phase I and Phase II forms of the disease [42].

Serological diagnosis of Q fever typically involves the use of an indirect immunofluorescence assay (IFA) with *C. burnetii* antigens. This test is performed on paired serum samples to identify a significant rise—typically fourfold or more—in antibody titers. Among serological methods, ELISA is generally favoured over IFA and the complement fixation test (CFT), especially in veterinary applications, due to its suitability for large-scale screening. Both CFT and ELISA offer improved sensitivity and specificity. However, CFT is considered less sensitive than ELISA or IFA. This method, a cold fixation microtechnique originally developed by Kolmer, is carried out using 96-well U-bottom microtiter plates and detects complement-fixing antibodies in the serum. The antigens used in this assay are a mixture of phase I and phase II strains, commonly derived from human isolates or the Nine Mile strain [42].

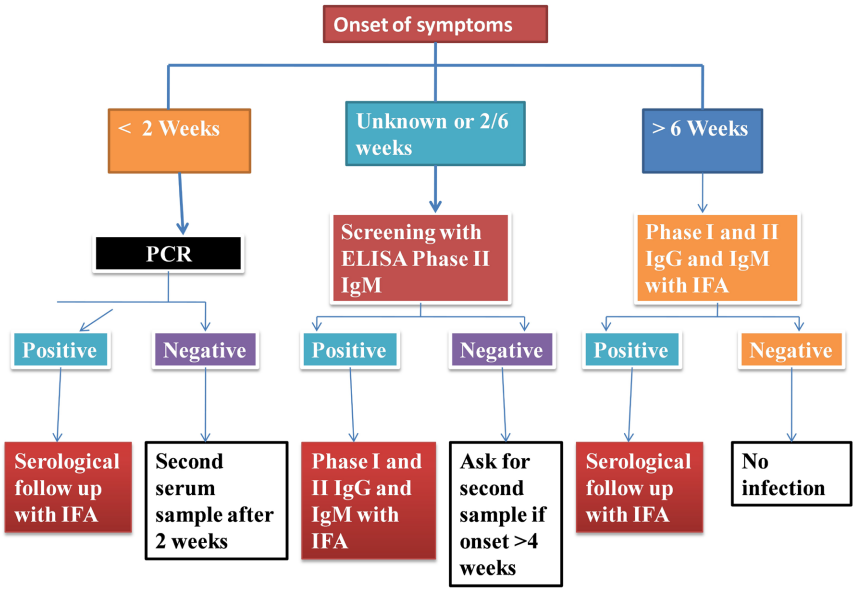
### 4.3. Molecular Diagnosis

Molecular diagnostics of Coxiella burnetii, the causative agent of Q fever, primarily utilise polymerase chain reaction (PCR) techniques, with real-time PCR being a standard method for detecting the bacteria's DNA in clinical samples. Advanced PCR methods, such as nested PCR and real-time PCR, enhance the detection accuracy of *C. burnetii*. Molecular diagnosis of *Coxiella burnetiid* can be performed on blood, placental tissue, animal faeces, and tick tissues using PCR. These methods are beneficial for diagnosing cases where traditional blood cultures are negative, such as in Q fever endocarditis. Polymerase chain reaction (PCR) offers high sensitivity and specificity for detecting acute Q fever in serum and tissue specimens. However, its effectiveness significantly decreases when using sputum or throat swab samples. Additionally, PCR demonstrates reduced sensitivity in cases of chronic Q fever. *Coxiella burnetii* DNA can be identified in blood samples for up to 17 days following the appearance of clinical symptoms [43].

For the diagnosis of Q fever, samples are analysed by PCR, and its amplification is carried out in two separate assays using different primer sets. Primers (approximately 24 bp) of the first set (1 F; 5′-GAGCGAACCATTGGT ATCG-3′ and 1 R; 5′-CTTTAACAGCGCTTGAACGT-3") and the second set (2 F; 5′-CGGGTTAAGCGTGCTCAGTATGTA-3" and 2 R; 5′-TGCCACCGCTTTTAATTCCTCCTC-3") were synthesized [43].

**Serological Analysis**

To resolve the issue community-acquired pneumonia related to Coxiella burnetii (Q fever) serological analysis is preferred to diagnose the samples. For serological analysis, serum samples are evaluated using phase II IgG and IgM enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) [44]. The PCR targeting the com1 and IS1111 genes was performed on blood samples. PCR amplicons were sequenced and phylogenetically analysed. Results among individuals with acute febrile illness showed that 34.7% (32 out of 92) were infected with *Coxiella burnetii*. PCR exhibited the highest sensitivity among the diagnostic methods employed. Complete genome analysis of *C. burnetii* will undoubtedly contribute to a better understanding of the pathogenesis of *C. burnetii* infection and improve Q fever diagnosis and immune prophylaxis [45]




## 4.4. Therapeutics

- **Use of broad-spectrum antibiotics**

Antibiotics work well against this disease's acute form, but as the infection develops into a chronic form, treatment becomes more difficult, and the illness frequently returns, which can result in a high death rate [46]. Antibiotic prophylaxis prevents endocarditis in 100% of patients at risk. Finally, IgG anticardiolipin antibodies, part of the autoimmune response during acute Q fever, have recently been added to the risk factors for endocarditis due to their ability to promote acute valvular lesions and endocarditis [8]. Acute Q fever in both children and adults is typically managed with doxycycline. In cases of chronic or localised persistent infection, a combination therapy—commonly doxycycline along with hydroxychloroquine—is recommended for effective treatment [47].

The presence of elevated phase II antibody titers within 1 - 3 weeks following symptom onset, along with the detection of IgM antibodies, indicates an acute

infection. In contrast, high phase I IgG antibody titers greater than 1:800, as determined by microimmunofluorescence, suggest a chronic *Coxiella burnetii* infection. For acute Q fever, a two-week course of doxycycline is recommended as the first-line treatment [8].

Current serological and molecular detection tools enable a reliable diagnosis of the disease. Culture of C. burnetii strains is mandatory to assess their susceptibility to antibiotics and sequence their genome, thereby optimizing patient management and epidemiological studies [48].

As a neglected zoonotic disease, limited data are available regarding the treatment of Q fever (*Coxiella burnetii* infection) in animals. Tetracycline, a broad-spectrum antibiotic, has proven effective in reducing the risk of abortion in ruminants infected with *Coxiella burnetii*. Its administration helps manage the infection and minimizes the chances of reproductive issues in affected animals [49].

### 4.5. Vaccine: Q-VAX

Various antigens, antibodies, and vaccines are employed to control *Coxiella burnetii* infection. Traditionally, whole-cell vaccines have been used to manage the disease. However, more recently, highly effective subunit, DNA-based, and multi-epitope vaccines have been developed. "Q-VAX", a whole-cell vaccine, is effective but has certain drawbacks, including the need for pre-vaccination screening and the risk of severe adverse reactions in individuals who have been previously exposed to the pathogen. The *C. burnetii vaccine, Q-Vax,* is based on the Henzerling strain. This vaccine, manufactured by CSL Limited in Parkville, Victoria, Australia, has been successfully used to protect individuals from Q fever infection. In Australia, the Q-Vax vaccine is registered and approved for use in humans but is recommended only for individuals over the age of 15 years. No vaccine has proven efficacy or afforded protection equivalent to that of Q-VAX in humans (see **Table 2**). To develop a successful vaccine, it is crucial to target the specific immune effectors required to eliminate the pathogen and induce immunological memory to combat it in subsequent encounters without establishing disease. Most Q fever vaccines developed so far can trigger an immune response, yet they do not provide effective protective immunity.

### 4.6. Subunit Vaccines

Subunit vaccines consist of immunodominant antigenic components that can be either recombinantly produced or purified from microbial sources. Researchers are working on developing a subunit vaccine using the outer membrane protein of *Coxiella burnetii*. The Com1 antigen, when formulated with TLR triagonists, has demonstrated both immunogenicity and acceptable reactogenicity in mouse models. The identification of immunodominant proteins, along with the discovery of novel antigens and careful selection of epitopes, plays a critical role in the development of effective subunit vaccines [50] (see **Table 2**).

The human Q fever vaccine Q-VAX exhibited certain limitations related to reactogenic responses in pre-sensitised individuals. Despite extensive efforts to develop next-generation human Q fever vaccines, only one vaccine, Q-Vax®, is commercially available. Q-Vax® is a phase I whole-cell vaccine, and its licensed use is limited to Australia, presumably due to the potential for a post-vaccination hypersensitivity response [51]. The development of fifth-generation vaccines with single antigenic molecules that display very fine antigenicity with reduced hypersensitivity is required [52]. Antibiotics work well against this disease's acute form, but as the infection develops into a chronic form, treatment becomes more difficult, and the illness frequently returns, which can result in a high death rate. The mechanisms driving reactogenic responses are still not fully understood, yet they are crucial considerations in the development of a safe and effective Q fever vaccine. This vaccine showed therapeutic efficacy in both untreated and treated patients. This also showed strong efficacy in high-risk human population groups.

The progression of vaccine development is examined, spanning from conventional whole-cell formulations to advanced subunit, DNA-based, and multi-epitope strategies. Emphasis is placed on cutting-edge technologies such as reverse vaccinology and immunoinformatics, which have facilitated the discovery of new antigenic targets. Recent clinical findings highlight the safety and immune-stimulating potential of next-generation vaccine candidates, along with key considerations for their production and deployment. Despite notable advancements in addressing the shortcomings of earlier vaccines, challenges persist in enhancing immunogenicity while maintaining safety across diverse population groups [19]. Subunit vaccines consist of key antigenic elements that are either produced through recombinant technology or isolated from microbial sources [53]. They offer the advantages of reduced reactogenicity and cost-effective production over traditional WCVs [54]. To augment immunogenicity, they are fused with a carrier or formulated with an appropriate adjuvant and administered in a multi-dose regimen [55]. A subunit vaccine composed of the outer membrane protein of *C. burnetii*, Com1 antigen formulated with TLR triagonist, was evaluated for immunogenicity and reactogenicity in a mouse model. Although it induced a strong IgG2c-skewed response, it only conferred partial protection against C. *Burnetii* compared to Q-VAX [56] (see **Table 2**). Fratzke *et al.* designed a subunit vaccine using six *C. burnetii* antigens formulated with multiple TLR agonists. Of the various vaccine formulations with different TLR agonists, only one vaccine containing TLR4, TLR7, and TLR9 agonists as triagonists demonstrated reduced reactogenicity and a comparable bacterial burden to WCVs [57]. The findings from these studies emphasize the use of an appropriate adjuvant for subunit vaccine development to induce the desired immune response required for *C. burnetii* clearance.

### 4.7. Coxiellosis Vaccines

Coxiellosis, caused of *Coxiella burnetii*, leads to abortion, infertility, and economic losses in livestock. These animals also serve as a reservoir for human Q

fever outbreaks, and direct transmission occurs through the consumption of animal products, insect bites, and the water route. Therefore, these animals can be vaccinated with inactivated whole-cell vaccines (WCVs), such as Coxevac (phase I) and Chlamyvax FQ (phase II). While WCVs have shown varying efficacy, phase I vaccines generally provide better protection, including reduced bacterial shedding and abortions, compared to phase II vaccines. However, adverse reactions such as injection site inflammation and fever are common. To address safety concerns, *Coxiella* membrane-based vaccines (CMR) are being explored as a less reactogenic alternative. To rapidly control the spread of disease in lactating dairy animals, vaccination is considered highly important. But it is a challenging job as *Coxiella burnetii* is also transmitted through, its DNA has been detected even in vaccinated animals. For instance, dairy goat milk has been found to contain *C. burnetii* DNA following immunization with Coxevac® [50] (see **Table 2**).

### Q-Fever Vaccine

*Coxiella burnetii* presents a unique challenge for vaccine development due to its obligate intracellular lifestyle, complex immune interactions, and poorly defined protective correlates. Phase I whole-cell vaccines (WCVs), including the licensed Q-Vax®, have provided effective prophylaxis since their early development in the 20th century. These vaccines demonstrated a synergistic interplay of multiple bacterial components, including phase I lipopolysaccharide (LPS) and structural proteins. For proper immunization, adjuvants are added to the antigen to induce potent and durable immunity. There is a problem in having purified specific antigenic determinants and immune pathways. Efforts have expanded to include modified WCVs, subunit vaccines, and LPS-mimic strategies. However, approaches that overlook the intricate host–pathogen interactions and immunological complexity of *C. burnetii* are unlikely to yield successful candidates. Continued progress will depend on deeper insights into *C. burnetii* virulence factors, antigenic targets, and the immune correlates of protection. Existing phase I whole-cell vaccines (WCVs), such as Q-Vax®, provide a valuable foundation for designing improved formulations. These vaccines are known for their robust immunogenicity, long-lasting immunity, and the convenience of a single-dose administration. However, next-generation vaccines based on pathogen particles, fragments of DNA, or mRNA may be more protective than the current standard.

For the quick resolution of the problem, the risk of post-vaccination hypersensitivity (PVH) reactions, as well as the need for pre-vaccination screening and biosafety concerns, should be characterized. An ideal next-generation Q fever vaccine would retain the immunogenic efficacy of phase I WCVs while minimising adverse reactions and simplifying production and administration protocols. (LPS)-Based vaccines may be safer and more effective Q fever vaccine [50] (see **Table 2**).

**Table 2.** Showing various types of Q fever vaccines, their characteristics, advantages and safety issues.

| Vaccine Type | Key Characteristics | Advantages | Safety issues | Current Status |
|---|---|---|---|---|
| Whole-Cell Vaccines (WCVs) | Inactivated or live attenuated vaccines (e.g., derived from Henzerling, Dyer strains); formaldehyde-inactivated or genetically modified versions | High protection (up to 95%), long-term efficacy (up to 5 years), strong immunity | Reactogenicity (local/systemic reactions), safety concerns with live forms | Licensed in some countries (e.g., Q-VAX in Australia); ongoing refinement efforts |
| Q-VAX The primary whole cell vaccine | Derived from the Henzerling phase I strain, formalin-inactivated vaccine. high efficacy | The whole primary cell vaccine for Q fever is a, with reported protection rates between 82% and 100%. | The efficacy of Q-VAX has been reported to be between 82 and 100% with long-lasting protection of up to 5 years. | While effective, it can be reactogenic in previously sensitized individuals, requiring pre-screening before vaccination |
| Subunit Vaccines immunodominant antigenic components | Composed of specific antigens (e.g., Com1, P1); produced using recombinant DNA technology; often formulated with adjuvants (e.g., TLR triagonists) | Safer than WCVs, targeted immune responses, scalable production | Variable protection, lower efficacy than WCVs, challenges in selecting effective antigen-adjuvant combos | Preclinical to early clinical stages |
| DNA Vaccines | Plasmid vectors encoding *C. burnetii* antigens; enhanced by electroporation, codon optimization | Good T-cell response, stable, non-infectious, prolonged antigen expression | Lower immunogenicity in humans, requires electroporation or delivery enhancement | Preclinical research phase |
| RNA Vaccines | Self-amplifying mRNA encoding Phase I antigens. formulated in lipid nanoparticles | Rapid development, high safety, strong immune response, no risk of infection | Stability and storage issues, early development phase | Promising in preclinical studies |
| Multi-Epitope Vaccines | Bioinformatically designed vaccines combining conserved B-cell and T-cell epitopes from multiple *C. burnetii* proteins | Broad immune coverage, rational design, balanced humoral and cellular immunity | Complex design and testing, variable response across populations | Experimental. early-stage development |
| Novel Delivery Systems & Adjuvants | Toll-like receptors bind lipid nanoparticles, and immune modulators, advanced adjuvants, mainly agonists | Enhanced antigen stability, uptake, and immune activation. extended shelf life | Need for optimization, cost of novel formulations | Integrated into next-generation vaccine designs |

### 4.8. Vector Control, Health and Hygiene

The most effective strategies for controlling and preventing coxiellosis include antibiotic therapy and vaccination. For acute Q fever in humans, doxycycline remains the preferred antibiotic. Preventive measures at the human level involve minimizing contact with animals or using protective gear such as gloves, boots, and masks when handling them. Proper pasteurization of milk- either at 145˚F (63˚C) for 30 minutes or 161˚F (72˚C) for 15 seconds is sufficient to eliminate *Coxiella burnetii* and other pathogens commonly found in raw milk. Maintaining strict hygiene, particularly in calving areas, is critical in reducing the risk of infection. Key practices include regular disinfection of calving pens, umbilical cord

sanitation, timely removal and safe disposal of aborted fetuses and fetal membranes and providing clean bedding during parturition. The immediate disposal of birth control products is essential to prevent their consumption by stray dogs, wild carnivores, or domestic animals, which can further contribute to environmental contamination and disease transmission. The health education of various occupational groups, such as abattoir workers, dairy farmers, shepherds, wool sorters, tanners and veterinary professionals about the source of infection, mode of transmission, severity of disease and personal hygiene should be imparted [58].

### 4.9. Precautions

Preventing the airborne transmission of Q fever involves measures such as avoiding the spread of manure during windy conditions and ensuring the proper disposal of contaminated materials. Consuming only pasteurized milk and dairy products is essential to reduce the risk of infection. In this context, raising public awareness and promoting education play a vital role in effective disease prevention. Farmers and farmyard people must keep livestock in a healthy and hygienic environment. Proper management of animal waste can reduce the concentration of *C. burnetii* in the environment. It is advised to immunize people who are occupationally exposed, including veterinary professionals, livestock handlers, and abattoir workers. The government should prioritize the vaccination of animals identified in endemic areas. Public awareness campaigns should emphasize the importance of using insecticides as an effective measure to control tick populations. Avoiding contact with animals, especially during pregnancy and childbirth, can reduce the risk of infection. Raising awareness about transmission routes, occupational risks, and preventive measures is crucial for public health.

### 4.10. Future Perspectives

For quick control of *C. burnetii* outbreaks, advanced diagnostic tools, techniques, and analytical tests with high sensitivity will be required before going for treatment options. Broad long-term control planning is necessary, particularly with multiple treatment options in animals, and practical approaches are needed for rapid prevention. A routine checkup of animals and the exposed human population, and vaccination is essential for fast management of this zoonotic disease in endemic areas. The *Coxiella vaccine* for animals is an effective means of minimizing disease transmission. It will be required in both types of animal herds, including those infected and those non-infected. Vaccination plays a crucial role in minimizing the re-emergence of *Coxiella burnetii* infections in both rural and urban areas. For timely and accurate diagnosis, it is essential to implement appropriate testing methods along with strain-specific identification and interpretation guidelines. All dairy animals should be vaccinated under field conditions to significantly reduce the risk of zoonotic transmission to humans. Additionally, establishing a safe distance between human residences and dairy farms is crucial to further reduce the likelihood of exposure to the pathogen.

## 5. Conclusion

Q fever is a globally significant infectious disease and is listed as a notifiable condition by the World Organization for Animal Health (WOAH). It holds considerable relevance for both public health and the economy. Sheep, cattle, and goats serve as the primary reservoirs of *Coxiella burnetii*, the rickettsia-like bacterium responsible for the disease, which occurs worldwide. Human infection typically results from inhaling contaminated aerosols, while arthropods do not play a role in their transmission. Chronic Q fever develops in less than 5% of infected individuals, often presenting as endocarditis or hepatitis, and may severely affect internal organs. The acute form of the disease is characterized by a sudden onset of fever, headache, fatigue, and interstitial pneumonitis, with symptoms often resembling those of influenza. In older or immunocompromised individuals, respiratory complications may be more pronounced. Bacterium *Coxiella burnetii* causes severe pathogenesis, resulting in deaths in both urban and rural areas worldwide. For the prevention and control of disease, earlier diagnosis is essential. Bacteria show genetic variations in different geographical regions and exhibit massive resistance to antibiotics. Diagnosis of Q fever typically involves basic methods such as serological testing, strain isolation, and polymerase chain reaction (PCR) analysis of clinical samples. Although Q fever can be prevented through vaccination, antibiotic treatment often shows limited effectiveness.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Meles, D., Mustofa, I., Wurlina, W., Rosyada, Z., Khairullah, A., Rachmawati, K., *et al.* (2025) Impact of Moringa Oleifera Leaf Extract on Renal Histopathology, Malondialdehyde, Superoxide Dismutase, and Calcium Oxalate Crystals in Rats (*Rattus norvegicus*) Exposed to Ethylene Glycol. *Open Veterinary Journal*, **15**, 1734-1746. https://doi.org/10.5455/ovj.2025.v15.i4.25

[2] Mazokopakis, E.E., Starakis, I.K., Papadomanolaki, M.G., Mavroeidi, N.G. and Ganotakis, E.S. (2013) The Hypolipidaemic Effects of Spirulina (*Arthrospira platensis*) Supplementation in a Cretan Population: A Prospective Study. *Journal of the Science of Food and Agriculture*, **94**, 432-437. https://doi.org/10.1002/jsfa.6261

[3] Georgiev, M., Afonso, A., Neubauer, H., Needham, H., Thiéry, R., Rodolakis, A., *et al.* (2013) Q Fever in Humans and Farm Animals in Four European Countries, 1982 to 2010. *Eurosurveillance*, **18**, Article ID: 20407. https://doi.org/10.2807/ese.18.08.20407-en

[4] Bielawska-Drózd, A., Cieślik, P., Mirski, T., Bartoszcze, M., Knap, J.P., Gaweł, J. and Żakowska, D. (2013) Q Fever—Selected Issues. *Annals of Agricultural and Environmental Medicine*, **20**, 222-232.

[5] Ullah, Q., Jamil, T., Saqib, M., Iqbal, M. and Neubauer, H. (2022) Q Fever—A Neglected Zoonosis. *Microorganisms*, **10**, Article 1530. https://doi.org/10.3390/microorganisms10081530

[6] Madariaga, M.G., Rezai, K., Trenholme, G.M. and Weinstein, R.A. (2003) Q Fever: A Biological Weapon in Your Backyard. *The Lancet Infectious Diseases*, **3**, 709-721. https://doi.org/10.1016/s1473-3099(03)00804-1

[7] Million, M. and Raoult, D. (2015) Recent Advances in the Study of Q Fever Epidemiology, Diagnosis and Management. *Journal of Infection*, **71**, S2-S9. https://doi.org/10.1016/j.jinf.2015.04.024

[8] Eldin, C., Mélenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., *et al.* (2017) From Q Fever to *Coxiella burnetii* Infection: A Paradigm Change. *Clinical Microbiology Reviews*, **30**, 115-190. https://doi.org/10.1128/cmr.00045-16

[9] Morroy, G., Keijmel, S.P., Delsing, C.E., Bleijenberg, G., Langendam, M., Timen, A., *et al.* (2016) Fatigue Following Acute Q-Fever: A Systematic Literature Review. *PLOS ONE*, **11**, e0155884. https://doi.org/10.1371/journal.pone.0155884

[10] Hanssen, D.A.T., Morroy, G., de Lange, M.M.A., Wielders, C.C.H., van der Hoek, W., Dijkstra, F., *et al.* (2019) Notification Data and Criteria during a Large Q-Fever Epidemic Reassessed. *Epidemiology and Infection*, **147**, e191. https://doi.org/10.1017/s0950268819000736

[11] Parker, N.R., Barralet, J.H. and Bell, A.M. (2006) Q Fever. *The Lancet*, **367**, 679-688. https://doi.org/10.1016/s0140-6736(06)68266-4

[12] Edouard, S., Courtois, G.D., Gautret, P., Jouve, J., Minodier, P., Noël, G., *et al.* (2016) High Prevalence of Mycoplasma Faucium DNA in the Human Oropharynx. *Journal of Clinical Microbiology*, **54**, 194-196. https://doi.org/10.1128/jcm.02068-15

[13] To, H., Sakai, R., Shirota, K., Kano, C., Abe, S., Sugimoto, T., *et al.* (1998) Coxiellosis in Domestic and Wild Birds from Japan. *Journal of Wildlife Diseases*, **34**, 310-316. https://doi.org/10.7589/0090-3558-34.2.310

[14] Ramatla, T., Khumalo, Z.T.H., Matshotshi, A., Lekota, K.E., Taioe, M.O. and Thekisoe, O. (2023) Molecular Detection of *Coxiella burnetii* and *Coxiella* Species in Rats and Chickens from Poultry Farms in North West Province, South Africa. *Veterinary Medicine and Science*, **9**, 2185-2191. https://doi.org/10.1002/vms3.1192

[15] Ferrara, G., Colitti, B., Pagnini, U., D'Angelo, D., Iovane, G., Rosati, S., *et al.* (2022) Serological Evidence of Q Fever among Dairy Cattle and Buffalo Populations in the Campania Region, Italy. *Pathogens*, **11**, Article 901. https://doi.org/10.3390/pathogens11080901

[16] Negi, M., Vergis, J., Vijay, D., Dhaka, P., Malik, S.V.S., Kumar, A., *et al.* (2015) Genetic Diversity, Virulence Potential and Antimicrobial Susceptibility of *Listeria monocytogenes* Recovered from Different Sources in India. *Pathogens and Disease*, **73**, ftv093. https://doi.org/10.1093/femspd/ftv093

[17] Sahu, R., Rawool, D.B., Dhaka, P., Yadav, J.P., Mishra, S.P., Kumar, M., *et al.* (2020) Current Perspectives on the Occurrence of Q Fever: Highlighting the Need for Systematic Surveillance for a Neglected Zoonotic Disease in Indian Subcontinent. *Environmental Microbiology Reports*, **13**, 138-158. https://doi.org/10.1111/1758-2229.12918

[18] Woldehiwet, Z. (2004) Q Fever (*Coxiellosis*): Epidemiology and Pathogenesis. *Research in Veterinary Science*, **77**, 93-100. https://doi.org/10.1016/j.rvsc.2003.09.001

[19] Raoult, D. and Marrie, T. (1995) Q Fever. *Clinical Infectious Diseases*, **20**, 489-496.

https://doi.org/10.1093/clinids/20.3.489

[20] Long, C.M., Beare, P.A., Cockrell, D.C., Larson, C.L. and Heinzen, R.A. (2019) Comparative Virulence of Diverse *Coxiella burnetii* Strains. *Virulence*, **10**, 133-150. https://doi.org/10.1080/21505594.2019.1575715

[21] Christodoulou, M. and Papagiannis, D. (2025) Q Fever Vaccines: Unveiling the Historical Journey and Contemporary Innovations in Vaccine Development. *Vaccines*, **13**, Article 151. https://doi.org/10.3390/vaccines13020151

[22] Rodolakis, A. (2009) Q Fever in Dairy Animals. *Annals of the New York Academy of Sciences*, **1166**, 90-93. https://doi.org/10.1111/j.1749-6632.2009.04511.x

[23] Anderson, A., Bijlmer, H., Fournier, P.E., Graves, S., Hartzell, J., Kersh, G.J., Limonard, G., *et al.* (2013) Diagnosis and Management of Q Fever—United States, 2013: Recommendations from CDC and the Q Fever Working Group. *The MMWR Recommendations and Reports*, **62**, 1-30.

[24] Benoit, M., Desnues, B. and Mege, J. (2008) Macrophage Polarization in Bacterial Infections. *The Journal of Immunology*, **181**, 3733-3739. https://doi.org/10.4049/jimmunol.181.6.3733

[25] Rath, M., Müller, I., Kropf, P., Closs, E.I. and Munder, M. (2014) Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages. *Frontiers in Immunology*, **5**, Article 532. https://doi.org/10.3389/fimmu.2014.00532

[26] Ka, M.B., Daumas, A., Textoris, J. and Mege, J. (2014) Phenotypic Diversity and Emerging New Tools to Study Macrophage Activation in Bacterial Infectious Diseases. *Frontiers in Immunology*, **5**, Article 500. https://doi.org/10.3389/fimmu.2014.00500

[27] Meghari, S., Honstettre, A., Lepidi, H., Ryffel, B., Raoult, D. and Mege, J. (2005) TLR2 Is Necessary to Inflammatory Response in *Coxiella burnetii* Infection. *Annals of the New York Academy of Sciences*, **1063**, 161-166. https://doi.org/10.1196/annals.1355.025

[28] Arango Duque, G. and Descoteaux, A. (2014) Macrophage Cytokines: Involvement in Immunity and Infectious Diseases. *Frontiers in Immunology*, **5**, Article 491. https://doi.org/10.3389/fimmu.2014.00491

[29] Elliott, A., Peng, Y. and Zhang, G. (2013) *Coxiella burnetii* Interaction with Neutrophils and Macrophages *in Vitro* and in SCID Mice Following Aerosol Infection. *Infection and Immunity*, **81**, 4604-4614. https://doi.org/10.1128/iai.00973-13

[30] Elliott, A., Schoenlaub, L., Freches, D., Mitchell, W. and Zhang, G. (2015) Neutrophils Play an Important Role in Protective Immunity against *Coxiella burnetii* Infection. *Infection and Immunity*, **83**, 3104-3113. https://doi.org/10.1128/iai.00042-15

[31] Gorvel, L., Textoris, J., Banchereau, R., Ben Amara, A., Tantibhedhyangkul, W., von Bargen, K., *et al.* (2014) Intracellular Bacteria Interfere with Dendritic Cell Functions: Role of the Type I Interferon Pathway. *PLOS ONE*, **9**, e99420. https://doi.org/10.1371/journal.pone.0099420

[32] de Winde, C.M., Munday, C. and Acton, S.E. (2020) Molecular Mechanisms of Dendritic Cell Migration in Immunity and Cancer. *Medical Microbiology and Immunology*, **209**, 515-529.

[33] Lazarevic, V., Glimcher, L.H. and Lord, G.M. (2013) T-Bet: A Bridge between Innate and Adaptive Immunity. *Nature Reviews Immunology*, **13**, 777-789. https://doi.org/10.1038/nri3536

[34] Mezouar, S., Lepidi, H., Omar Osman, I., Gorvel, J., Raoult, D., Mege, J., *et al.* (2020)

T-Bet Controls Susceptibility of Mice to *Coxiella burnetii* Infection. *Frontiers in Microbiology*, **11**, Article 1546. https://doi.org/10.3389/fmicb.2020.01546

[35] Ledbetter, L., Cherla, R., Chambers, C., Zhang, Y., Mitchell, W.J. and Zhang, G. (2020) Major Histocompatibility Complex Class II-Restricted, $CD4^+$ T Cell-Dependent and -Independent Mechanisms Are Required for Vaccine-Induced Protective Immunity against *Coxiella burnetii*. *Infection and Immunity*, **88**, e00824-19. https://doi.org/10.1128/iai.00824-19

[36] Casadevall, A. and Pirofski, L. (2003) Antibody-Mediated Regulation of Cellular Immunity and the Inflammatory Response. *Trends in Immunology*, **24**, 474-478. https://doi.org/10.1016/s1471-4906(03)00228-x

[37] Casadevall, A. and Pirofski, L. (2006) A Reappraisal of Humoral Immunity Based on Mechanisms of Antibody-Mediated Protection against Intracellular Pathogens. *Advances in Immunology*, **91**, 1-44. https://doi.org/10.1016/s0065-2776(06)91001-3

[38] Zhang, Y., Zhang, G., Hendrix, L.R., Tesh, V.L. and Samuel, J.E. (2012) *Coxiella burnetii* Induces Apoptosis during Early Stage Infection via a Caspase-Independent Pathway in Human Monocytic THP-1 Cells. *PLOS ONE*, **7**, e30841. https://doi.org/10.1371/journal.pone.0030841

[39] Brindha, S., Shinde, S.V., Bhure, M., Chaudhari, S.P., Khan, W.A., Kurkure, N.V., *et al.* (2024) Occurrence of Coxiellosis in Ruminants and Its Associated Risk Factors. *Acta Tropica*, **255**, Article ID: 107235. https://doi.org/10.1016/j.actatropica.2024.107235

[40] Dabaja, M.F., Greco, G., Villari, S., Vesco, G., Bayan, A., Bazzal, B.E., Ibrahim, E., Gargano, V., Sciacca, C., Lelli, R., Ezzedine, M., Mortada, H., Tempesta, M. and Mortada, M. (2019) Occurrence and Risk Factors of *Coxiella burnetii* in Domestic Ruminants in Lebanon. *Comparative Immunology, Microbiology & Infectious Diseases*, **64**, 109-116. https://doi.org/10.1016/j.cimid.2019.03.003

[41] Meles, D., Khairullah, A., Mustofa, I., Wurlina, W., Akintunde, A., Suwasanti, N., *et al.* (2024) Navigating Q Fever: Current Perspectives and Challenges in Outbreak Preparedness. *Open Veterinary Journal*, **14**, 2509-2524. https://doi.org/10.5455/ovj.2024.v14.i10.2

[42] Sundar, B., Shinde, S.V., Dongre, S.A., Chaudhari, S.P., Khan, W.A., Patil, A.R., *et al.* (2024) Acute Q Fever in Individuals with Acute Febrile Illness & Exposure to Farm Animals: Clinical Manifestations & Diagnostic Approaches. *Indian Journal of Medical Research*, **159**, 681-688. https://doi.org/10.25259/ijmr_1549_23

[43] Kazar, J. (2005) *Coxiella burnetiid* Infection. *Annals of the New York Academy of Sciences*, **1063**, 105-114. https://doi.org/10.1196/annals.1355.018

[44] Schack, M., Sachse, S., Rödel, J., Frangoulidis, D., Pletz, M.W., Rohde, G.U., *et al.* (2013) *Coxiella burnetii* (Q Fever) as a Cause of Community-Acquired Pneumonia during the Warm Season in Germany. *Epidemiology and Infection*, **142**, 1905-1910. https://doi.org/10.1017/s0950268813002914

[45] Yandiola, P.P.E., Capelastegui, A., Quintana, J., Diez, R., Gorordo, I., Bilbao, A., *et al.* (2009) Prospective Comparison of Severity Scores for Predicting Clinically Relevant Outcomes for Patients Hospitalized with Community-Acquired Pneumonia. *Chest*, **135**, 1572-1579. https://doi.org/10.1378/chest.08-2179

[46] Kovácová, E. and Kazár, J. (2000) Rickettsial Diseases and Their Serological Diagnosis. *Clinical Laboratory*, **46**, 239-245.

[47] Francis, R., Mioulane, M., Le Bideau, M., Mati, M., Fournier, P., Raoult, D., *et al.* (2020) High-Content Screening, a Reliable System for *Coxiella burnetii* Isolation from Clinical Samples. *Journal of Clinical Microbiology*, **58**, e02081-19.

https://doi.org/10.1128/jcm.02081-19

[48] Sam, G., Stenos, J., Graves, S.R. and Rehm, B.H.A. (2023) Q Fever Immunology: The Quest for a Safe and Effective Vaccine. *npj Vaccines*, **8**, Article No. 133. https://doi.org/10.1038/s41541-023-00727-6

[49] Long, C. (2021) Q Fever Vaccine Development: Current Strategies and Future Considerations. *Pathogens*, **10**, Article 1223. https://doi.org/10.3390/pathogens10101223

[50] Heidary, M., Kaviar, V.H., Shirani, M., Ghanavati, R., Motahar, M., Sholeh, M., *et al.* (2022) A Comprehensive Review of the Protein Subunit Vaccines against Covid-19. *Frontiers in Microbiology*, **13**, Article 927306. https://doi.org/10.3389/fmicb.2022.927306

[51] Moyle, P.M. and Toth, I. (2013) Cheminform Abstract: Modern Subunit Vaccines: Development, Components, and Research Opportunities. *ChemInform*. https://doi.org/10.1002/chin.201322240

[52] Chen, S., Pounraj, S., Sivakumaran, N., Kakkanat, A., Sam, G., Kabir, M.T., *et al.* (2023) Precision-Engineering of Subunit Vaccine Particles for Prevention of Infectious Diseases. *Frontiers in Immunology*, **14**, Article 1131057. https://doi.org/10.3389/fimmu.2023.1131057

[53] Gilkes, A.P., Albin, T.J., Manna, S., Supnet, M., Ruiz, S., Tom, J., *et al.* (2020) Tuning Subunit Vaccines with Novel TLR Triagonist Adjuvants to Generate Protective Immune Responses against *Coxiella burnetii*. *The Journal of Immunology*, **204**, 611-621. https://doi.org/10.4049/jimmunol.1900991

[54] Fratzke, A.P., Jan, S., Felgner, J., Liang, L., Nakajima, R., Jasinskas, A., *et al.* (2021) Subunit Vaccines Using TLR Triagonist Combination Adjuvants Provide Protection against *Coxiella burnetii* While Minimizing Reactogenic Responses. *Frontiers in Immunology*, **12**, Article 653092. https://doi.org/10.3389/fimmu.2021.653092

[55] Pal, M., Zende, R., Bekele, A., Rebuma, T., Panicker, N. and Made Dwi Mertha Adnyana, I. (2025) Bacteriophage Therapy against Antibiotic-Resistant Bacteria: A Critical Appraisal. *American Journal of Public Health Research*, **13**, 67-73. https://doi.org/10.12691/ajphr-13-2-5

[56] National Association of State Public Health Veterinarians National Assembly of State Animal Health Officials (2013) Prevention and Control of *Coxiella burnetii* Infection among Humans and Animals: Guidance for a Coordinated Public Health and Animal Health Response, 2013.

[57] Sterling, T.R., Villarino, M.E., Borisov, A.S., Shang, N., Gordin, F., Bliven-Sizemore, E., *et al.* (2011) Three Months of Rifapentine and Isoniazid for Latent Tuberculosis Infection. *New England Journal of Medicine*, **365**, 2155-2166. https://doi.org/10.1056/nejmoa1104875

[58] Hogerwerf, L., van den Brom, R., Roest, H.I.J., Bouma, A., Vellema, P., Pieterse, M., *et al.* (2011) Reduction of *Coxiella burnetii* Prevalence by Vaccination of Goats and Sheep, the Netherlands. *Emerging Infectious Diseases*, **17**, 379-386. https://doi.org/10.3201/eid1703.101157