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Review Article

Coxiella burnetii Pathogenesis: Emphasizing the Role of the Autophagic Pathway

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Abstract

Coxiella burnetii (*C. burnetii*), the etiological agent of the Q fever disease, ranks among the most sporadic and persistent global public health concerns. Ruminants are the principal source of human infections and diseases present in both acute and chronic forms. This bacterium is an intracellular pathogen that can survive and reproduce under acidic (pH 4 to 5) and harsh circumstances that contain *Coxiella*-containing vacuoles. By undermining the autophagy defense system of the host cell, *C. burnetii* is able to take advantage of the autophagy pathway, which allows it to improve the movement of nutrients and the membrane, thereby extending the vacuole of the reproducing bacteria. For this method to work, it requires the participation of many bacterial effector proteins. In addition, the precise and prompt identification of the causative agent of an acute disease has the potential to delay the onset of its chronic form. Moreover, to make accurate and rapid diagnoses, it is necessary to create diagnostic devices. This review summarizes the most recent research on the epidemiology, pathogenesis, and diagnosis approaches of *C. burnetii*. This study also explored the complicated relationships between *C. burnetii* and the autophagic pathway, which are essential for intracellular reproduction and survival in host cells for the infection to be effective.

Keywords: Autophagy pathway, *Coxiella burnetii*, *Coxiella*-containing vacuoles, Epidemiology, Intracellular replication, Pathogenesis, Q fever

1. Context

Coxiella burnetii (*C. burnetii*) is a gram-negative, intracellular pathogen that primarily infects alveolar macrophages (AMs) and has evolved mechanisms to survive in acidic, degradative, and harsh phagolysosome environments. *C. burnetii* is the causative agent of Q fever which has caused worldwide pandemic outbreaks. The development of extracellular culture medium and remarkable breakthroughs in genetic modification techniques have allowed us to acquire a better grasp of *C. burnetii* pathogenesis (1). Unlike other intracellular bacteria that evolve into phagolysosome-like compartments via the endocytic route, *C. burnetii* enters the host cells, resides there, and replicates in a membrane-bound vacuole. Acidification is necessary to begin *Coxiella*-containing vacuole (CCV) formation and the bacterial effector translocation to the host cell via the type IVB secretion system (T4BSS). While the CCV has lysosomal properties, it also has unique traits, such as homotypic fusion, a cholesterol-rich limiting membrane, and an intensive autophagosome interaction. Many *Coxiella* vacuolar proteins (Cvps) have been localized to endosomal compartments to divert autophagy and

establish an intracellular replication habitat for *C. burnetii*, and CCVs synthesis requires autophagy, which is triggered by these proteins. For example, CvpB and autophagy are obviously necessary for CCV homotypic fusion (2).

2. Evidence Acquisition

Understanding the features and intracellular pathogenic process of *C. burnetii* may help in the treatment and control of this enigmatic pathogen. Therefore, this study aimed to provide a comprehensive overview of infections, epidemiology, pathogenesis aspects, and the development of axenic cultures for viable pathogen cultivation and isolation. This study also discusses the complicated connection of *C. burnetii* with the autophagic pathway, which is critical in its replication and escape from host immune systems.

3. Results

3.1. Infections

C. burnetii is an environmentally-stable bacterium with the lowest infectious dose known to humankind and just 10 bacteria capable of causing illness in a healthy human (3). This bacterium is transmitted to humans through the inhalation of contaminated dust particles or aerosols arising from contaminated soil, as well as contact with infected animal products. Farm animals (particularly cattle, sheep, and goats), pets, and ticks are the most important global reservoirs that can preserve infectious organisms alive for long periods (4, 5). *C. burnetii* is designated as a category B organism with a biological threat that may be misapplied as biological warfare because of its great resistance to harsh conditions, high infectivity, and ease of spreading to humans (6).

Q fever is a common zoonotic illness with subclinical, acute, and chronic manifestations in humans. Patients develop a high temperature, sweating, nausea, vomiting, and diarrhea after an incubation period. *C. burnetii* can spread to the blood during the acute phase, and fever can remain for more than one week or less than three weeks (7). Endocarditis is the most prevalent chronic clinical presentation in people with valvulopathy or immunocompromised immune systems. However, the less common chronic Q fever symptoms include hepatitis, osteomyelitis, vasculitis, recurrent fever, and interstitial lung fibrosis (Figure 1) (8). Furthermore, fever alone is insufficient to establish the presence of chronic infection; a microbiological criterion should be specified, in addition to clinical or organic/lesional determinants. At the same time, despite seroconversion, the majority of people (60%) remain asymptomatic (7). The advancement of diagnostic procedures and developments in imaging technologies allow the exact identification of infectious foci centers and lead clinicians to a more effective therapeutic strategy. The phrase "chronic Q fever" is becoming outdated when new criteria are introduced. According to some research, seroconversion is a strain-dependent event. As a result of these factors, the disease's incidence rate is substantially lower than the positive findings of serological testing. Therefore, this shift is critical in evaluating chronic diseases (9). Historically, acute and chronic Q fever were distinguished by phase II and phase I antibody profiles against lipopolysaccharide (LPS) and protein antigens. High antibody titers against phase II were considered to signify acute Q fever, and antibodies against phase I were a prognostic indication of chronic Q fever. Three pieces of evidence challenged this traditional paradigm. The first was the discovery of high and persistent phase I antibodies in acute infections induced by certain strains. Second, a poor serological response was linked to a persistent *C. burnetii* infectious focus. Therefore, the French Q fever National Reference Center revealed that 5% of individuals with chronic, targeted infections had a low IgG titer of less than 1/800 during a 25-year period (1991-2016). Finally, without an infectious outbreak, persistent Q fever fatigue syndrome was associated with a positive Q fever serology test. The Dutch consensus Q fever group thus developed a new nosology of chronic Q fever in 2012 and divided chronic forms into three categories, including possible,

probable, and proven cases of chronic Q fever (10). Only phase I IgG levels larger than 1:1.024 indicated the possibility of persistent Q fever in the absence of additional clinical manifestations. A positive PCR for *C. burnetii* or serological criteria, in the presence of endocarditis or vascular infections, is used to diagnose chronic Q fever. The occurrence of clinical circumstances ranging from pregnancy to immunosuppression or granulomatous lesions with serological findings (1:1,024 for *C. burnetii* phase I IgG) indicates the existence of probable chronic Q fever (11).

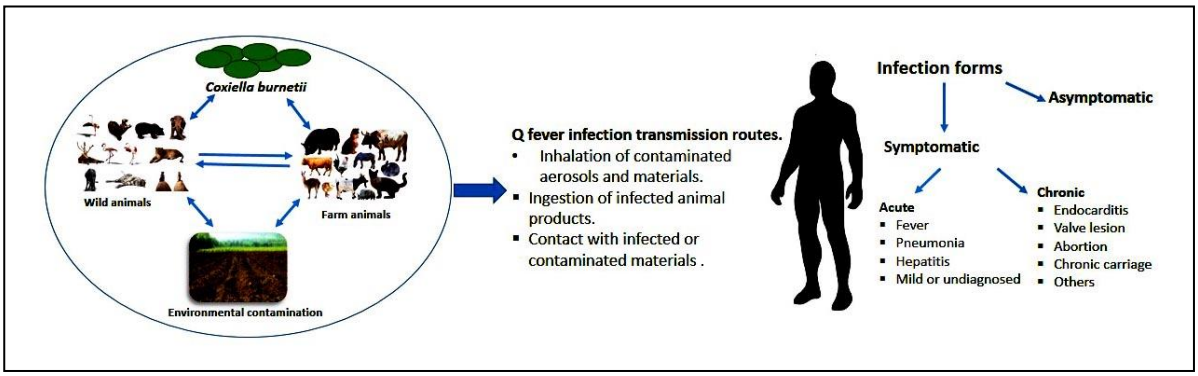

Figure 1. Overview of the natural cycle of *C. burnetii* infections and how they are transmitted from the environment to humans, as well as the clinical aspects of these infections

3.2. Epidemiology

The global incidence and prevalence rates of Q fever are poorly established due to varied symptoms, misdiagnoses, and wide geographical distribution. A healthy immune system makes it possible to combat acute Q fever, resulting in fewer diagnoses and reports of its prevalence. Acute Q fever affects around 50 out of every 100,000 people each year, while chronic Q fever affects one out of every million (12). The risk of *C. burnetii* infection increases in warmer climates, during the summer months, as well as in farms, slaughterhouses, or research laboratories which study and manipulate infected tissues or desired pathogens (9-11). Increased exposure rates frequently correspond with an increased risk of outbreaks in the affected region. The reservoirs of *C. burnetii* include a diverse range of vertebrate and invertebrate hosts; nevertheless, cattle, sheep, and goats are the principal reservoirs. Some tick species have also been reported to host *C. burnetii*, indicating that these species might be *C. burnetii* reservoirs. It has also been proposed that amoebae offer suitable conditions for the development and survival of *C. burnetii* under unfavorable environmental settings (10). Infectious *C. burnetii* particles have also been found in chicken products, milk, urine, and feces of infected animals. Q fever is mostly spread by the inhalation of aerosolized bacteria, and it is seen in endemic or epidemic forms in many geographical regions (Figure 1) (13). This disease is most common in France, Spain, Australia, and the United States. Over three years, large-scale outbreaks have been reported in the Netherlands, with 4,000 cases correlated with a rise in the overall population of goats on farms and in the proximity of urban areas (13). Furthermore, these Q fever epidemic outbreaks have been linked to the importation of infected animals, a poor level of immunization, and a lack of continuous surveillance programs in this population (13). Q fever was widespread in Africa, with seropositivity rates ranging from 1% to 16% recorded in various locations. A high positive serology rate was associated with the largest density of domestic ruminants in these

locations. The precise frequency of Q fever is understated due to the restricted availability of diagnostic instruments. Furthermore, seroprevalence investigations in Egypt have revealed that camels are major reservoirs of the Q fever disease (14).

3.3. Pathogenesis

C. burnetii strains are the primary cause of acute and chronic forms of Q fever with distinct pathotypes, according to phylogenetic analysis. The pathogenicity and virulence of *C. burnetii* are determined by the kind of infected animal species, the *C. burnetii* strain, the route of infection, and the pathogen inoculum size. Aerosolization is the standard approach for instilling *C. burnetii* pathogenesis in animal models (mice and guinea pigs) (15). Comparing Nine Mile phase I (NMI) to Priscilla phase I, the inoculum of less than four NMI organisms might produce seroconversion and fever. At the same time, more than 10^5 Priscilla phase I bacteria are required to elicit symptoms via the intraperitoneal route. Furthermore, Priscilla-infected guinea pigs have a delayed onset of symptoms, a decreased bacterial load in the spleens, and a slower disease progression. Based on the *in vivo* model infection, two epidemic strains, including Guiana and Netherlands-like strains, were more virulent than the NM strain. Netherlands-like strains have intermediate virulence among the two strains mentioned (15). *C. burnetii* strains have a chromosome with 1,989,565 to 2,214,254 base pairs that encodes a collection of adhesion, invasion, detoxification, and secretion system proteins. According to preliminary research, *C. burnetii* encodes secretion systems that are extremely similar to *Legionella pneumophila*'s defect in organelle trafficking/intracellular multiplication (Dot/Icm) machinery (16). *C. burnetii* not only prefers certain phagocytic cells (particularly AMs), but it also invades and multiplies inside a wide range of cell types (2). Following aerosol transmission, pathogens infiltrate professional phagocyte cells via RAC1-dependent phagocytosis. It enters the host cell passively via actin-dependent phagocytosis (v3 integrin, as the primary receptor), which is implicated in actin-cytoskeletal ruffling. Purified LPS of *C. burnetii* phase I can induce membrane ruffling on the host cell surface (17). Furthermore, the rearrangement of the filamentous (F)-actin cytoskeleton is only observed in phase I monocytes. This membrane ruffling is likewise dependent on TLR4 expression on the host cell surface. The invasion of a host cell using v3 integrin is not related to inflammation induction and immune system activation on the invading cell (18). The bacterial ligand for v3 integrin is yet to be adequately characterized in phagocytic cells. In contrast, the OmpA protein in non-professional phagocyte cells has been identified as a bacterial invasin that enables active entrance into the host cells via a zipper-like mechanism (19). *C. burnetii* tissue culture experiments revealed that phases I and II have different uptake kinetics, and avirulent phase II is readily absorbed into the host cell. Two bacterium phases, on the other hand, have the same replication rate and can create identical count CCVs (20). Nascent CCVs mature into the early phagosome in a regulated sequential event. It obtains the small GTPase RAB5 (which causes the nascent phagosome to acidify to pH 5.4), the microtubule-associated protein light-chain 3 (LC3), and the early-endosomal marker protein EEA1. The late phagosome then acquires the GTPase RAB7, the lysosome-associated membrane glycoprotein 1 (LAMP1), LAMP2, and the vacuolar ATPase, and then the luminal pH is reduced to around 5. The acquisition of hydrolases, cathepsins, and the vacuolar ATPase by the fusion of lysosomal compartments results in a pH drop of roughly 4.5. Unlike the traditional endosomal route, CCVs grow and fill the whole host cell cytoplasm between eight hours and two days after infection. A two-hour interval in CCV growth and lysosomal enzyme buildup after infection may allow small cell variants (SCVs) to convert to large cell variants (LCVs). *C. burnetii* replicates within this membrane-bound compartment, known as a "parasitophorous vacuole (PV)", and may withstand lysosomal hydrolases while

utilizing the acidic pH for metabolic activation. Furthermore, PV interacts with secretory and autophagic processes implicated in *C. burnetii* replication. The type T4SS is a multi-protein complex that has been divided into two systems: type IVA (T4ASS) and type IVB (T4BSS), based on its similarity to the *Agrobacterium tumefaciens* and *Legionella pneumophila* (*L. pneumophila*) systems, respectively (21). *C. burnetii* has a T4BSS system similar to the Dot/Icm secretion system of *L. pneumophila* that acts as a surrogate host for the production of putative *C. burnetii* T4SS effectors, which is critical for *C. burnetii* intracellular survival (21). In *C. burnetii*, 24 homologs of Dot/Icm secretion components were found. Nonetheless, three components were missing: DotJ, DotV, and IcmR, and it appears that the duplication of other homologous genes can complete the function of the T4BSS systems in *C. burnetii*. T4BSS and T4ASS share the core transport complex (DotC, DotD, DotF, DotG, and DotH) which connects the inner and outer membranes. T4BSS secretion machinery function is implicated in DotB protein ATPase activity, but its mechanism of action is unknown (21, 22). Professional phagocytes (monocytes and macrophages) are the host cells that are colonized by phagocytosis (specific receptor-ligand interactions). *C. burnetii*'s environmental survival and stability have been related to the shift between SCVs, which are metabolically inactive and resistant, and LCVs, which are metabolically active and sensitive (2). The main virulence factor of *C. burnetii* is LPS, a substantial component of its outer membrane that contributes to immunological hijacking and pathogenic characteristics. *C. burnetii* antigen variants have been identified as phase I (smooth form) and phase II (rough form) (rough form). The phase I form has an intact and full-length LPS and can survive inside monocytes and macrophages. This form has been identified in natural sources as a virulent form of *C. burnetii*. Avirulent or phase II strains, on the other hand, arise after several passages of phase I in embryonated eggs or cell culture conditions without immune system interference (23, 24). *C. burnetii* phase II displays irreversible modifications in LPS structures and the production of a shortened LPS. In other words, the atypical structure of the LPS of *C. burnetii* strains may weaken and impair host cell immune defense (25). The difference between the virulent (phase I) and avirulent (phase II) forms of *C. burnetii* is based on the presence of a full-length O-antigen LPS in the virulent *C. burnetii* with saccharidic units containing peculiar sugars, l-virenose, dihydrohydroxystreptose, and galactosamine uronyl-α-(1, 6)-gluco. In addition, phase II LPS lacks the outer core, compared to phase I or intermediate LPS (23). Both virulent and avirulent *C. burnetii* lipid A molecules have the same ionic species and fragmentation properties in mass spectrometry, showing that their structures are extremely similar and possibly identical. The core polysaccharide in the virulent and avirulent *C. burnetii* LPS is conserved and comprises a heptasaccharide in the proximal domain of lipid A. Two terminal d-mannoses (Man), 2- and 3,4-linked d-glycero-d-manno-heptoses and terminal 4- and 4,5-linked 3-deoxy-d-manno-oct-2-ulosonic acid residues, combine to produce the heptasaccharide (26). Furthermore, the LPS-protein complex extracted from phase I has a low potential to stimulate the immune system and is employed as a vaccination against Q fever (25). It is worth noting that a third *C. burnetii* LPS has been found as an intermediate-length LPS on the surface of NM Crazy strains, as determined from guinea pig placental tissues (23). These avirulent *C. burnetii* NM and NM Crazy strains have large chromosomal deletions (23). These deletions eliminate open reading frames involved in the biosynthesis of O-antigen sugars, including the rare sugar virenose. The description of the virenose biosynthesis pathway proposes the formation of GDP—D-virenose via the addition of a methyl group at position C3" and perhaps the open

reading frame CBU0691, as well as the inversion of the stereochemistry at position C2" (26).

3.4. Subversion of Autophagy by *C. burnetii*

Macroautophagy, commonly known as autophagy, is a well-preserved cellular mechanism involved in the degradation and recycling of intracellular components, such as misfolded proteins, lipids, malfunctioning organelles, other macromolecules, and entrapped microorganisms (26-28). This degradation pathway was primarily used for cellular homeostasis since it creates the autophagosome (a double-membrane vesicle compartment) and recycles small-molecule compounds to supply energy to the cells. An autophagosome is a double-membrane vesicle that transports cytoplasmic material to lysosomes. Autophagy occurs at high levels under some circumstances, such as nutritional stress and bacterial invasion; nevertheless, it occurs at low levels in normal physiological cell conditions. Therefore, autophagy serves not only to destroy faulty macromolecules or organelles but also to provide protection against a variety of intracellular pathogens (28). Autophagy occurs in three forms: non-selective, chaperone-mediated, and selective. Excessive or unwanted cytoplasmic materials are extensively digested in non-selective forms to prepare the energy for the cells. Selective autophagy, on the other hand, is defined by identifying and targeting certain cargo molecules. It has been classified into lipophagy (lipid components), aggrephagy (proteins), mitophagy (mitochondria), lysophagy (lysosomes), pexophagy (peroxisomes), glycophagy (glycogen), ribophagy (ribosomes), ER-phagy (endoplasmic reticulum), and xenophagy (invading pathogens) terms. Xenophagy is a highly conserved eukaryotic cellular mechanism that involves a collection of proteins in targeting the invading pathogens. Xenophagy begins with identifying the invading pathogens and directing them to the phagophore (28).

Following that, the phagophore expands and elongates, resulting in the creation of the autophagosome. Autophagosomes mature into autolysosomes by fusion with the lysosome organelle, and autolysosome contents are degraded utilizing lysosomal enzymes (Figure 2) (29). However, several bacteria have developed a number of strategies for manipulating the host autophagy process to avoid this defensive response and live and proliferate in the host cell cytoplasm. *C. burnetii* was found to be one of these bacteria that survived and replicated within AM cells via the xenophagy pathway (30). *C. burnetii* remains in endosome vesicles after internalization and goes through the host endosomal network, but it does not impede the fusion of the pathogen-containing phagosome with lysosomes. The acidification of the phagosome activates T4SS on *C. burnetii*, facilitating the formation of highly fusogenic CCVs (31). The induction of the autophagy pathway in Chinese hamster ovary cells increased the number of infected cells with *C. burnetii*, the bacterial load, and the size of the CCVs, according to Gutierrez, Vázquez (29). Furthermore, they claimed that activating and using the autophagy pathway improves *C. burnetii* replication and viability (29). Another study discovered that autophagosomes fuse with CCVs in a clathrin heavy chain dependent way whose expression and autophagy in CCVs have a substantial relationship (30). Larson, Sandoz (32) also showed that *C. burnetii* inhibits the mammalian (or mechanistic) target of rapamycin complex 1 (mTORC1) kinase and that the infected host cells have increased the production of LC3 and p62 transcription factors, as well as TFE3 protein. They also claimed that the *C. burnetii* suppression of mTORC1 is needed for the intracellular reproduction of this pathogen. Several Cvps have been found in endosomes and CCVs, diverting autophagy and preparing a favorable intracellular replication environment for *C. burnetii*. These proteins are crucial in modifying the autophagy pathway to induce autophagy for *Coxiella* vacuole formation, as well as supplying sufficient nutrients for *C. burnetii* replication and the expansion and maturation of CCV compartments (Table 1) (28). The function of the major proteins, in conjunction with the intracellular proliferation of *C. burnetii*, is summarized below.

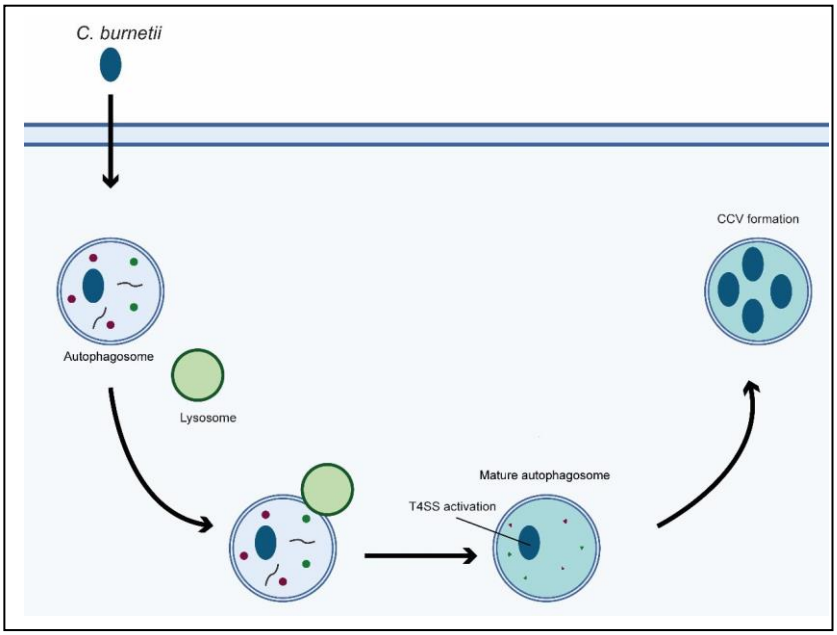

Figure 2. The schematic intracellular proliferation of *C. burnetii*

Table 1. *C. burnetii* effector proteins in the intracellular proliferation

Virulence effector	Mechanism	Reference
Coxiella Vacuolar Protein A (CvpA)	Mechanism	(33)
Coxiella Vacuolar Protein B (CvpB)	Interacts with AP2, clathrin-mediated endocytosis (CME) adaptor that has been required for CHC accumulation at the CCVs.	(28)
Coxiella Vacuolar Protein C (CvpC)	Maintain LC3 on the CCVs membranes to aid fusion with autophagosomes.	(34)
Coxiella Vacuolar Protein D (CvpD)	Associated to the CCVs and required for its expansion.	(35)
Coxiella Vacuolar Protein E (CvpE)	Associated to the CCVs and required for its expansion.	(28)
Coxiella Vacuolar Protein F (CvpF)	Associated to the CCVs and required for its expansion.	(28)
Cig57 (CBU1751)	Interactions with Rab26 are required for *C. burnetii*-induced LC3 lipidation, CCVs formation, and LC3 delivery to the CCVs.	(30)

Cig57 (CBU1751) is a 48.8 kDa effector protein that is co-regulated with *icm* genes and is involved in intracellular replication in *C. burnetiic*. Transposon mutagenesis disrupting the Cig57 protein considerably impacted bacterial growth rate in host cells, as well as CCV biogenesis and morphology, resulting in a small CCV phenotype (36). This effector protein regulates and interacts with clathrin-mediated endocytosis in association with FCHO2 protein and other clathrin adaptor proteins. It has many endocytic sorting (MES) motifs (two dileucine motifs and one tyrosine motif) (37, 38). FCHO2 is a clathrin accessory protein that curves the plasma membrane to create clathrin-coated vesicles in *C. burnetii* endocytosis and is a Cig57 binding partner (39). This protein is present in the subcellular membrane and is not required for CHC accumulation at CCVs. Furthermore, LC3B autophagosomes are essential for CHC concentration, and Cig57 was shown to be required to transfer LC3B positive vesicles to the CCVs, permitting LC3 lipidation and sequestosome-1 accumulation. *C. burnetii* takes use of endocytic pathways as a source of membrane for CCV growth and maturation in the host cell by using Cig57 and FCHO2 (30, 33, 38). Cig57 enhances LC3B lipidation and clathrin localization at CCVs during intracellular *C. burnetii* infections.

3.4.1. *Coxiella* Vacuolar Protein F (CvpF)

The regulating Rab GTPases are GTP-bound in the active state and GDP-bound in the inactive state. By interacting with other partner proteins, these regulatory proteins influence vesicular trafficking and contribute to autophagosome formation (40). CvpF (CBU0626) is a Cvp protein that is essential for intracellular replication and CCV production in *C. burnetii* (19). In the severe combined immunodeficiency disease mouse model, Siadous, Cantet (41) studied the pathophysiology of a CvpF mutant strain. According to the findings, this strain exhibits substantial replication attenuation and a reduced capacity to produce inflammation (splenomegaly) within the spleen. When compared to the wild-type strain, vacuole production was inhibited in this mutant strain, demonstrating that CvpF is involved in CCV formation by influencing the autophagy process.

Furthermore, the ectopic expression of CvpF could cluster and remodel the endosomal compartment in the transfected cells (41). CvpF has an internal endocytic sorting motif that interacts with adaptor complexes (APS) and LC3B through an unknown mechanism (33). This effector protein interacts with RAB26 GTPase protein, and the inactivation or depletion of RAB26 from host cells is associated with rebate CCV size and a reduction in LC3B recruitment to CCV compartments. Moreover, CvpF has also been shown to interact with the inactive RAB26 form, promoting transport to endosomal vesicles and accumulation at CCVs (42). It is a hypothesis that CvpF acts as the Guanine Exchange Factor or GDI Dissociation Factor for RAB26 and agitates LC3B lipidation through ATG16L1 protein in autophagy flux in transfected cells. Therefore, CvpF interacts with Rab26 and induces the accumulation and lipidation of LC3B at the CCVs (41).

3.4.2. *Coxiella* Vacuolar Protein A (CvpA)

The whole-genome sequence analysis of the *C. burnetii* NM strain revealed that the open reading frame CBU0665 encoded a protein related to T4BSS effector proteins, comprising LRR domains (eukaryotic-like leucine-rich repeat) and MES motifs identified by the AP2 adaptors and clathrin (43). This protein is called *Coxiella* vacuolar protein A (CvpA) or Cig18, which has been involved in the subversion of clathrin-coated vesicle trafficking in infected cells. It also has a function in *C. burnetii* intracellular growth, which is required for PV maturation (33). *C. burnetii ΔcvpA* mutant strain has been dramatically reduced in intracellular replication and produces smaller CCVs than the wild-type strain in the THP-1 macrophage cell line (33, 44). The ectopic expression investigation of mCherry-CvpA indicated a close colocalization with clathrin in the plasma membrane, the EEA1 on the pericentrosomal vesicles in uninfected cells, and also the CCV membrane in the infected cells (36). Furthermore, mCherry-CvpA inhibits clathrin-mediated endocytosis of transferrin molecules, and, in addition, the depletion of clathrin or AP2 with siRNA significantly harms intracellular replication and CCV biogenesis (33). Collectively, this evidence proposes a critical function for CvpA in *C. burnetii* growth and CCV biogenesis via the subversion of clathrin-mediated vesicular trafficking of the host cells.

3.4.3. *Coxiella* Vacuolar Protein B (CvpB)

One of the main intracellular replication aspects of the *C. burnetii* is the high fusogenicity of CCVs in shaping a single large CCV, as well as hijacking and exploiting endolysosomal trafficking, secretory, apoptosis, and autophagy pathways of the host cell to create and maintain a favor replicative niche (36). CvpB protein (also known as Cig2) is a 93.1 kDa effector protein that interacts with phosphatidylinositol 3-phosphate (PI3P) and phosphatidylserine on vacuolar structures. (45). Larson, Beare (34) demonstrated that that *C. burnetii ΔcvpB* strain reduced capability to replicate in THP-1 human macrophage cells. This matter was linked to the disability of the homotypic fusion of CCVs and resulted in the generation of multi-PV phenotype in Vero cells. Another study described that the mutation of the *cig* gene used transposon insertion mutants to establish a multi-PV growth phenotype in infected cells without intracellular growth

defects (16). This observation implies that CvpB is required for the fusion and biogenesis of the large CCV. The *C. burnetii* CvpB mutant-infected Galleria mellonella (G. mellonella, wax moth) model indicated that the wax moth was more tolerant of the CvpB mutants, and that CvpB mutant replication was unaffected by infection with HeLa cells. Additionally, G. mellonella CvpB mutant demonstrated a slower death rate than wild type (WT); however, both showed the same replication rate (46). Similarly, introducing the RavZ effector (which inhibits autophagy by cleaving lipidated LC3) into *C. burnetii* results in a multi-PV phenotype, implying that autophagosomes interact with the mature CCV and form an autolysosomal environment for it. CvpB was shown to inhibit the PI3P 5-kinase *PIKfyve*, which phosphorylates PI3P to produce PI(3,5)P2. As expected, siRNA silencing *PIKfyve* restored the multi-PV phenotype observed in CvpB transposon mutants (45).

3.5. Diagnosis Approach

Laboratory workers are still at significant risk of infection from clinical and animal materials due to the highly contagious nature of *C. burnetii*. The detection of *C. burnetii* in animals and livestock, especially asymptomatic ones, is critical to restrict the spread of the pathogen to the human population (47). Mice and guinea pigs were injected with phase I for pathogen isolation. Phase II was, however, isolated using cell cultures or yolk sacs (1). Molecular and serological techniques are widely used to diagnose Q fever in entities. The detection of this pathogen requires a biosafety level three setting and an extended incubation time (47). A combination of microbiological and organic/lesional criteria is necessary to diagnose Q fever accurately.

Seroconversion, which occurs six weeks after the beginning of early symptoms and is a common occurrence in cases of *C. burnetii* infection, is the primary diagnostic value. Being precise and straightforward, the indirect immunofluorescence antibody test is often regarded as the gold standard for serological assays. Variations in the applied technique among reference laboratories make it challenging to establish a cutoff point for the serological test. Because of this, it is vital to implement additional tests, such as the Western blot, to distinguish between acute Q fever and past infections (48). In ruminant placentas, lymphoma tissues, and cardiovascular tissue samples, fluorescent in situ hybridization has been used to detect the *C. burnetii* genome (49). Two distinct repeating sequences, IS1111 and IS30a, are widely used to detect *C. burnetii* in various samples because those have multiple copies in the genome, and the lyophilization technique may improve *C. burnetii* identification (50). As previously stated, *C. burnetii* is a microaerophilic, fastidious, and slow-growing intracellular bacterium that requires acidic parasitophorous CCV conditions to be isolated. The L929 cell line (a murine fibroblast cell line) is widely used for *in vitro* experiments. In addition to the cell culture medium, the axenic medium was developed to isolate and cultivate clinical and environmental samples and genomic manipulation of the pathogen. Clinical examination (for detecting infectious foci), transthoracic echocardiography (for identifying valvulopathy and distinguishing acute from persistent Q fever), and 18F-FDG-PET/CT-scan imaging are also performed to diagnose Q fever precisely and accurately. To read more, see the paper by Melenotte, Million (1) for diagnostic specifics and further information.

4. Conclusions

C. burnetii, the zoonotic agent of Q fever, remains a severe public health concern, and it has the potential to trigger outbreaks in ruminant livestock and human beings globally. The majority of human infections originated from contaminated domestic ruminant materials. Phylogenetic studies revealed that distinct *C. burnetii* pathotypes could establish acute and chronic disease, and a similar genotype has been found to have identical multi-locus VNTR analysis and single-

nucleotide polymorphism types. Additionally, this similarity was observed at genome and proteome levels to be genotype-specific. This may imply the clonal origin and genotype-specific evolution of strains. The discovery of the cell-free culture medium demonstrated that this pathogen preferred microaerophilic conditions, and its cultivation enabled further exploration of virulence pathogenesis mechanisms and gene mutagenesis studies. As an intracellular pathogen, *C. burnetii* can tolerate unfavorable CCVs environment by exploiting and subverting the host cells' autophagy process and encoding several Cvps to subvert the host autophagy defense mechanism and establish a favorable replication niche and supply nutrient resources.

Authors' Contribution

Study concept and design: A. A.
Acquisition of data: M. K., J. A. and Z. M.
Analysis and interpretation of data: M. K., J. A. and Z. M.
Drafting of the manuscript: A. A., M. K., J. A. and Z. M.
Critical revision of the manuscript for important intellectual content: A. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

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