

# *Coxiella burnetii*: living inside the host cell

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**Abstract:** *Coxiella burnetii* is an obligate intracellular pathogen and the causative agent of Q fever. In this brief review, we describe how recently described mechanisms help our understanding of *C. burnetii* invasion and its survival in the host cell by the formation of a replicative niche: the *Coxiella*-containing vacuole. We describe the actin-associated proteins involved in the internalization of *C. burnetii*, and we discuss the contribution of diverse degradation pathways of the cell during the formation and stabilization of the *Coxiella*-containing vacuole.

## Introduction

*Coxiella burnetii* is a Gram-negative bacterium belonging to the Gamma subdivision of the Proteobacteria. Morphologically, it is a highly pleomorphic coccobacillus (0.2-0.4 µm wide and 0.4-1 µm long) (Maurin and Raoult, 1999). It shows a biphasic developmental cycle in which goes through a small-cell variant (SCV) and a large-cell variant (LCV) (Wiebe *et al.*, 1972; Howe and Mallavia, 2000; Sandoz *et al.*, 2014). SCVs are the infectious form because they are more stable to environmental conditions than LCVs, and are also highly resistant to physical and chemical stresses (McCaul and Williams, 1981; Bielawska-Drózd *et al.*, 2014).

After Vero cell infection, the change from the SCV towards the LCV begins in early stages (2 h post-infection), being the LCV the prevailing form after the first day post-infection (Coleman *et al.*, 2004; Howe and Mallavia, 2000). The LCV is metabolically active and has an active type 4B secretion system (T4BSS) that is essential for the formation of the intracellular niche of the bacteria. The LCV transforms back to the SCV during the stationary phase of intracellular growth that starts approximately 6 days post-infection (Coleman *et al.*, 2004). Homogeneous populations of SCVs are observed in infected Vero cells and in axenic acidified citrate cysteine medium 2 (ACCM2) after a prolonged incubation period (21-28 days) (Sandoz *et al.*, 2014). As different species of the genus *Brucella*, *C. burnetii* can undergo a transition of its lipopolysaccharide (LPS) from “smooth” to “rough” with loss of its virulence (Rittig *et al.*, 2003; Mancilla, 2016). In the particular case of *C. burnetii*,

the virulent strain called Nine Mile I (NMI), after several passages of cell lines, loses part of its LPS and becomes avirulent for immunocompetent animals (Nine Mile II or NMII) (Moos and Hackstadt, 1987; Hoover *et al.*, 2002).

## Q fever

In 1937, Derrick described a febrile illness in workers from Brisbane, Australia (Derrick, 1983). When he inoculated guinea pigs with blood from patients, they developed a disease similar to that seen in humans. After that, Burnet observed *Rickettsia*-like microorganisms in the liver of infected guinea pigs (Burnet and Freeman, 1983) and Cox could culture these microorganisms in embryonated eggs (Cox and Bell, 1939). The causative agent of this febrile disease was named *Coxiella burnetii* in honor of the discoverers, Cox and Burnet. Initially, this disease was called “fever of the slaughterers” and “Queensland rickettsial fever”, but now it is known as “Q fever” in humans and “coxiellosis” in animals.

This disease is a zoonosis spread throughout the world (Poppe, 1950), but because it does not abruptly affect human health or the production of domestic animals, the geographical distribution has not been well established. Cases of Q fever have been reported in at least 50 countries in different parts of the world (Woldehiwet, 2004). Several cases have been reported in New Zealand (Greenslade *et al.*, 2003), a country considered free of this disease (Hilbink *et al.*, 1993). *C. burnetii* *R. rickettsii*, *R. massiliae* and *R. parkeri* have been detected in Argentina using serological assays (Cicuttin *et al.*, 2015). More recently, a case of *C. burnetii* infection has been found in the region of Chile (Weitzel *et al.*, 2016), and four cases in the Brazilian region (Siciliano *et al.*, 2015).

Q fever is considered a respiratory disease, which is mainly acquired inhaling aerosols or contaminated dust. The incidence is high in people who are in contact with animals,

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such as veterinarians and slaughterers, because the infected animals spread the causative agent through their fecal matter, urine, milk, and birth products. Especially, the amniotic fluid and the placenta have a large amount of microorganisms that can be aerosolized or dried and thus contaminate the soil (Hellenbrand *et al.*, 2001; Tissot-Dupont *et al.*, 1999).

Due to its high resistance, *C. burnetii* can remain virulent for several months in dried products and, under certain circumstances, it can spread by the wind, so a direct contact with infected animals is not an essential condition for infection (Tissot-Dupont *et al.*, 2004). Because of these and other characteristics, *C. burnetii* has been classified as a potential bioterrorist agent (Oyston and Davies, 2011).

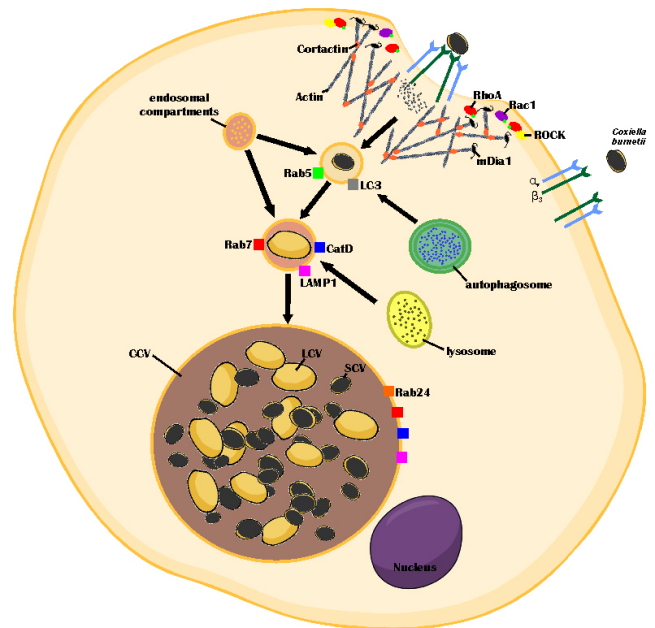
In animals, the most important route for pathogen entry is the inhalation of contaminated aerosols; nevertheless, the infection can also be transmitted by pets that have been bitten by an infected tick (Duron *et al.*, 2015). Infected ticks are probably the most important animals in maintaining the natural infective cycle; however, a cycle can involve mice, cats, and humans, besides ticks (Kazar, 2005). Also, infection with *C. burnetii* is usually asymptomatic; in the acute phase, the presence of the bacteria can be demonstrated in the blood, lungs, spleen, and liver. *C. burnetii* infection often becomes chronic, with persistent shedding of *C. burnetii* in feces and urine. However, animals do not develop chronic endocarditis as that observed in humans. In females, the uterus and mammary glands are the primary sites of infection (Maurin and Raoult, 1999). The only pathological manifestations that have associated with chronic *C. burnetii* infection in animals is abortion, mainly in sheep and goats, and lower birth weight and infertility in cattle (Aitken, 1989).

In humans, infection with *C. burnetii* causes clinical manifestations that are highly variable, ranging from an acute syndrome to a fatal chronic infection. The most common form of chronic Q fever is endocarditis. Typically, it is a disseminated disease often associated with multi-organ involvement including chronic hepatitis. Less frequently, there have been described chronic infections of vascular aneurysms or prosthesis chronic osteomyelitis and osteoarthritis, lung tumors, pulmonary fibrosis, and chronic hepatitis, with no accompanying endocarditis (Fergusson *et al.*, 1985). The incubation period of the disease ranges from 1 to 3 weeks and after that 50-60% of the patients are asymptomatic. There have been described nearly 30 different clinical syndromes in patients that present symptoms. The disease is like flu, manifested by fever, sweat, coughs, myalgia and arthralgia. Without treatment, the fever persists for 1-3 weeks or, in some cases, longer. A high percentage of patients have also pneumonia and hepatitis. Pneumonia is frequently moderate, but progression to acute respiratory distress syndrome can also occur. In the case of presenting hepatitis, the patients can show a moderate increase in the level of transaminases (Hartzell *et al.*, 2008).

### Entry of *C. burnetii* into the host cells

*C. burnetii* is an obligate intracellular pathogen that presents tropism by monocytes and alveolar macrophages (Fernandes *et al.*, 2016); however, it can infect different cell types such as epithelial cells (Sobotta *et al.*, 2017). Sobotta *et al.* (2017) postulated that the epithelial tissues could form a secondary

site of infection used as a niche to avoid the host immune system. Initial studies indicated that *C. burnetii* NMII is internalized more efficiently than its virulent variant NMI by human monocytes (Capo *et al.*, 1999). However, Shannon and Heinzen (2008) showed that both strains infect and replicate in macrophages derived from human monocytes with similar extent and kinetics. In support of these observations, a recent report demonstrates that the two forms of *C. burnetii* invade with the same efficiency human alveolar macrophages, but some differences were observed in macrophages derived from bovine monocytes (Sobotta *et al.*, 2016).



**FIGURE 1. Description of the internalization process of *Coxiella burnetii* and the biogenesis of the *Coxiella*-containing vacuole.** *C. burnetii* adheres to the host cell through  $\alpha_v\beta_3$  integrin receptors and induces its uptake by an actin-dependent mechanism that involves actin-interacting proteins like cortactin, RhoA, Rac1, ROCK, and mDia1. Once internalized in the host cell, the bacterium resides in a compartment called “*Coxiella*-containing vacuole (CCV)”. During the intracellular trafficking, *C. burnetii* interacts with different endosomal-autophagosomal compartments. In the first stage of the process, the CCV acquires Rab5 and the autophagosomal marker LC3 (microtubule-associated protein light-chain 3). The nascent CCV progressively matures losing Rab5 and obtaining Rab7, LAMP1 (lysosome-associated membrane glycoprotein 1) and lysosomal enzymes, such as CatD (cathepsin D). During the development of the CCV, the bacterium changes from the SCV towards the LCV allowing the beginning of its replication. Then, the CCV increases its size to occupy most of the cellular cytoplasm. The acquisition of additional membranes from other intracellular compartments could also contribute to create this spacious CCV.

The entry of *C. burnetii* to the host cell is a passive, actin-dependent phagocytosis (Tujulin *et al.*, 1998). It has been observed that live or dead bacteria are internalized with similar efficiency in fibroblasts and macrophages (Baca *et al.*, 1993; Tujulin *et al.*, 1998). Bacteria with a deficient secretion system are capable of invading different cell types (Beare *et al.*, 2011). Martinez *et al.* (2014) determined the presence of

an invasion in the outer membrane of *C. burnetii*, namely OmpA (outer membrane protein A), that increases the internalization levels in non-phagocytic cells. The genome sequencing of the NMI (Seshadri *et al.*, 2003) and NMII (Millar *et al.*, 2017) strains allowed to postulate other possible bacterial molecules potentially involved in their adherence and internalization. These include proteins with RGD domains, repeated ankyrin motifs and molecules similar to EnhA/B/C of *Legionella pneumophila* that enhance its entry to host cells (Seshadri *et al.*, 2003).

Different components of the host cell play a key role in the adhesion and internalization of *C. burnetii*. Interestingly, alveolar macrophages can produce C-type lectin surfactant protein D, which binds to *C. burnetii* and reduces the attachment and phagocytosis of the bacteria (Soltysiak *et al.*, 2015). The adhesion of the bacteria requires molecules that must be present in the host membrane since it decreases due to the treatment of the cell with different proteases (Baca *et al.*, 1993). The receptors involved in the internalization of *C. burnetii* in monocytes/macrophages are the  $\alpha_v\beta_3$  and  $\alpha_M\beta_2$  integrins (Capo *et al.*, 1999). Different works show the participation of host cell membrane cholesterol in the *C. burnetii* entry by  $\alpha_v\beta_3$  receptors and postulate the possible importance of “lipid rafts” in the process (Howe and Heinzen, 2006; Gilk *et al.*, 2013). The  $\alpha_M\beta_2$  integrins are excluded from the plasma membrane that contacts the virulent *C. burnetii*. Interestingly, this interaction induces large cell membrane protrusions (Capo *et al.*, 1999). Similar membrane rearrangements have been observed during the interaction of Toll-like receptor 4 with *C. burnetii* NMI through its LPS (Meconi *et al.*, 2001). Nevertheless, the internalization of the avirulent strain NMII is highly efficient with discrete modification of the host cell membrane and the microfilaments (Capo *et al.*, 1999).

Membrane modifications of the host cell depend on rearrangements of the actin cytoskeleton (Zamboni *et al.*, 2004; Conti *et al.*, 2014). Microfilaments play an essential role in the infection of both strains of *C. burnetii* (Baca *et al.*, 1993). In our laboratory, we have demonstrated that GTPases of the Rho family regulate the initial stage of the *C. burnetii* internalization of RhoA acting through their effectors mDia1 and ROCK (Salinas *et al.*, 2015). Interestingly, cortactin, an actin regulatory protein, together with the Src kinase signaling pathway, participate in the internalization of the NMII strain by epithelial cells (Rosales *et al.*, 2012).

#### Intracellular traffic of *C. burnetii*

After being internalized, *C. burnetii* resides into vacuoles that transit along the phagocytic pathway to fuse with the lysosomal compartment. Through time, these vacuoles fuse with each other and with other intracellular compartments to form a large vacuole called “*Coxiella*-containing vacuole (CCV)”. The intracellular itinerary of *C. burnetii* was established by the sequential acquisition of Rab5 and Rab7 (Berón *et al.*, 2002; Romano *et al.*, 2007; Ghigo *et al.*, 2009), early and late endocytic markers, respectively, and the lysosomal enzymes phosphatase and cathepsin D (Howe and Mallavia, 2000). The interaction of CCVs with lysosomes is significantly slower than that observed in typical phagosome maturation (Li *et al.*, 2008; Vieira *et al.*, 2002). Interestingly,

the newly formed CCVs recruit the autophagosomal marker LC3, which also suggests the interaction with the autophagy pathway at a very early stage of infection (Gutierrez *et al.*, 2005; Romano *et al.*, 2007). This interaction persists at later post-infection times. The delay to arrive to lysosomes and the interaction with the autophagy pathway, which provides membrane, nutrients, and metabolites, for the intracellular life of *C. burnetii* as it increases its replication (Gutierrez *et al.*, 2005; Romano *et al.*, 2007; Howe and Mallavia, 2000). It has been speculated that delaying maturation favors bacterium differentiation from the metabolically inactive SCV to the metabolically active LCV and thus resists the deleterious effects when receiving the degradative lysosomal machinery (Larson *et al.*, 2016).

The active role of *C. burnetii* during its intracellular journey is closely related to bacterial proteins secreted by its Type 4 secretion system (Voth and Heinzen, 2009). In a final stage, the CCV acquires a large size using endosomes, lysosomes, autophagosomes, and other intracellular compartments as membrane sources (Campoy *et al.*, 2011; Campoy *et al.*, 2013). The CCV contains flotillin 1 and 2 (Howe and Heinzen, 2006) and the autophagosomal markers LC3 (Berón *et al.*, 2002; Romano *et al.*, 2007) and Rab 24 (Gutierrez *et al.*, 2005), Rab 7 (Romano *et al.*, 2007; Berón *et al.*, 2002; Ghigo *et al.*, 2009), the lysosomal glycoproteins LAMP-1, LAMP-2 and LAMP-3 (Voth and Heinzen, 2007), and the H<sup>+</sup>-ATPase. One of the outstanding characteristics of the CCV is its high fusogenicity. The CCV can fuse with endosomes loaded with dextran (Maurin *et al.*, 1992; Heinzen *et al.*, 1996), with phagosomes loaded with zymosan particles, and with latex beads (Veras *et al.*, 1994). Surprisingly, the CCV fuses with vacuoles containing other pathogens such as *Mycobacterium avium* (De Chastellier *et al.*, 1999; Gomes *et al.*, 1999), *M. tuberculosis* (Gomes *et al.*, 1999), *Salmonella enterica* (Drecktrah *et al.*, 2007), *Leishmania amazonensis* (Veras *et al.*, 1994) or *Trypanosoma cruzi* (Andreoli *et al.*, 2006). Obviously, there is no doubt about the sociability of *C. burnetii* in the “intracellular world”.

The characterization of the molecular machinery involved in the interplay between the host cell and *C. burnetii* could provide knowledge to understand the mechanisms of infectious diseases and contribute to the design of adequate therapeutic strategies to attenuate the virulence of this pathogen.

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