**Coxiella burnetii** infection (Q fever) in humans and animals: a general overview


**ABSTRACT**

*Coxiella burnetii*, a ubiquitous intracellular bacterial pathogen, is the causative agent of zoonotic disease known as Q fever, with acute and chronic clinical manifestations. This bacterium is able to infect a wide range of animals, but cattle, sheep and goats are the principal reservoirs. It has been reported in many countries including Asian subcontinent like Bangladesh and India. Inhalation of contaminated aerosols is the main transmission route for humans. The spectrum of clinical manifestations in humans and animals are diverse, ranging from self-conversion without any clinical symptoms to fatal consequences. The acute infection in humans manifests as self-limiting febrile illness, pneumonia, or hepatitis, whereas endocarditis is the major manifestation in chronic cases. Infection in domestic animal is usually asymptomatic and remains unrecognized, but it may cause abortion, especially in sheep and goat. Detection of high serum antibody titer or bacterial DNA from the aborted material or from milk and urine are the basis of confirmatory diagnosis of *C. burnetii* infection. Doxycycline and tetracyclines are the recommended antibiotic for humans. Cotrimoxazole and rifampin are the drugs of choice for the patients allergic or contradicted to tetracyclines. Oxytetracycline in the last trimester of pregnancy is usually recommended for animals. Control of Q fever in humans is largely dependent upon the control of infection in animals. Several preventive measures were suggested and have shown effective to prevent and reduce the animal and environmental contamination. Vaccination of animals against *C. burnetii* found to be effective for preventing abortion.

**Keywords:** *Coxiella burnetii*, Q fever, Review.

**INTRODUCTION**

*Coxiella burnetii* (*C. burnetii*), the causative agent of Q fever in humans and animals, is an obligate gram-negative intracellular bacterium of the family *Coxiellaceae* (Angelakis and Raoult, 2010). This bacterium can infect a wide range of hosts, but cattle, sheep and goats are the principal reservoirs (Angelakis and Raoult, 2010). Q fever is a zoonosis, and domestic ruminants such as cattle, sheep, and goats are considered the primary source of human infections (Maurin and Raoult, 1999; Angelakis and Raoult, 2010). *C. burnetii* infection can produce both acute and chronic forms of the disease in humans. A self-limiting febrile condition is the most frequent manifestation in most cases (Tissot-Dupont and Raoult, 2007). Spontaneous abortion, intrauterine foetal death, premature delivery or retarded intrauterine growth may occur in pregnant women (Carcopino et al., 2007). Mortality is a rare outcome of the acute form of the disease. The major clinical manifestation of chronic form of Q fever is endocarditis (Tissot-Dupont and Raoult, 2007) with case fatality in untreated cases exceeding 10% (Maurin and Raoult, 1999). Beside zoonotic importance, *C. burnetii* also produce health and production problems in domestic ruminants including cattle. Infection in cattle usually remains unrecognized (Rodolakis, 2009), but it causes sporadic reproductive problems such as abortion, infertility and mastitis (Barlow et al., 2008). These illustrate that *C. burnetii* infection does induce economic losses in dairy herds. Q fever was first reported amongst abattoir workers in Brisbane, Australia in 1935 as an outbreak of febrile illness and was code-named “Q” for query fever (Derrick, 1937). However, McDade (1990) stated that Queensland was the state in which the
disease described first and thus, named as Q fever, where the Q stood for Queensland. The aetiologic organism of Q fever was first isolated by Burnet and Freeman (1937) and named it Rickettsia burnetii. It was found morphologically similar to other gram-negative bacteria. Afterwards, based on its cultural and biochemical characteristics, it was reclassified as a member of a new genus Coxiella (Philip, 1948). The genus “Coxiella” was named after Herald R. Cox, who first isolated this microorganism in the United States (Philip, 1948).

Q fever has a worldwide distribution and is endemic in many European countries (ECDC, 2010; Frankel et al., 2011). It is considered as an emerging or re-emerging disease in countries such as the Netherlands and France (Frankel et al., 2011; Roest et al., 2011b). It is enlisted in the multiple species disease category in the OIE (World Organization for Animal Health) and is one of the 47 enlisted communicable diseases within the EU legal framework on communicable disease surveillance and notification (Commission Decision 2000/96/EC) (Paul, 2013). In the European Union, Q fever/C. burnetii infection is also included in list B disease (other zoonoses and zoonotic agents that shall be monitored according to the epidemiological situation in a MS) in Annex I of Directive 2003/99/EC. But, it is not included in the notifiable disease list in domestic ruminants in the EU legislation (Directive 82/849/EEC) or in Regulation 2160/2003/EC for zoonosis control options (Paul, 2013). In the Netherlands, the emergence of Q fever was notified in humans in 2007 with a peak occurrence in 2009, despite the fact that the disease was considered endemic for several decades (Roest et al., 2011b). Q fever in humans and animals has also been reported from countries of Asian subcontinents like India (Vaidya et al., 2010). Hence, it is expected that C. burnetii infection is also present in animals in Bangladesh as unregulated cross-border animal transport exist between India and Bangladesh. Indeed, Haider et al., (2015) reported serological evidence of C. burnetii infection in cattle and goats from Bangladesh. Although officially not acknowledged, C. burnetii infection is apparently prevalent at least in cattle and goat population in Bangladesh and the disease host, vector and risk factors favourable for this infection are available in this geographical area. However, due to its apparently invisible clinical and socioeconomic impact, very little or no attention in some instance has been paid to explore the biology, epidemiology, prevention and control measures of C. burnetii infection. The recent outbreak of Q fever in the Netherlands and other EU countries reminds us that this disease is capable of producing similar impact in any favorable naïve geographical area. This review article presents a brief overview of C. burnetii infection (Q fever) with special emphasis on its bacteriology, and pathogenesis, epidemiology (distribution, transmission, reservoir and risk factors), clinical manifestations and diagnosis of infection and disease, treatment, general prevention and control measures, and finally some concluding remarks.

Bacteriology

C. burnetii is a non-motile obligate intracellular bacterium. This pleomorphic rod shaped organism possesses a cell membrane similar to that of other Gram-negative bacteria (Maurin and Raoult, 1999). Although Gram-negative, it cannot be stained by the Gram staining method. However, Gimenez’s method is used (Gimenez, 1965). Stamp, Macchiavello, modified Ziehl–Neelsen method and modified Koster, can also be used to stain bacteria (Maurin and Raoult, 1999). C. burnetii was classified as a member of the order Rickettsiales, family Rickettsiaceae, the tribe Rickettsiae and the genera Rickettsia. The bacterium was reclassified into the order Rickettsiales, based on 16S rRNA sequence analysis and phylogenetic studies (Maurin and Raoult, 1999). It now belongs to the gamma subdivision of the Proteobacteria.
After internalisation, *C. burnetii*, like *Ehrlichiae*, remains within a parasitophorous vacuole (PV) of infected cells and completes all of its life cycle stages within this vacuole (McCaul and Williams, 1981). A high number of bacteria are generated within the PV, and replicate at an estimated doubling time of 20-45 h (Mertens and Samuel, 2007). Electron microscopy shows that the organism has two metabolic forms: 1) the small-cell variant (SCV) is a compact, small rod and has a very electron-dense centre of condensed nucleoid filament; and 2) the large-cell variant (LCV) which is comparatively larger and less electron-dense than the metabolically active intracellular form of *C. burnetii*. Unlike other members of the tribe *Rickettsiae*, *C. burnetii* is highly resistant to physical and chemical exposure, and it can form a small structure similar to an endospore within the infected cell (McCaul and Williams, 1981). The antigenic variation (phase variation) of the bacterium is determined by lipopolysaccharides (LPS) (Angelakis and Raoult, 2010). Phase I bacteria (wild virulent type) with a smooth full length LPS were isolated from infected humans, animals and arthropods (Maurin and Raoult, 1999). Phase I bacterium converts to an avirulent phase II with rough LPS after several passages in embryonated egg or cell cultures (Hotta et al., 2002). However, virulent phase II, an intermediate phase with semi-rough LPS, has been described in some of the studies (Amano et al., 1987; Angelakis and Raoult, 2010). Based on the restriction fragment length polymorphism (RFLP), strains of *C. burnetii* are grouped into six (I-VI) genomic groups (Maurin and Raoult, 1999). The virulence and the pathogenicity of the *C. burnetii* are associated with genetic characteristics, plasmid groups and type of strains (Hendrix et al., 1991), and also with host factors such as pregnancy (Woldehiwet, 2004). Plasmid groups I, II and III are responsible for acute infection, whereas groups IV and V cause chronic infection (Hendrix et al., 1991).

**Pathogenesis**

The pathogenesis of *C. burnetii* infection in humans and animals is not clearly understood. But, it is believed that bacterial LPSs play an important role in the pathogenesis of Q fever in both humans and animals (Angelakis and Raoult, 2010, Maurin and Raoult, 1999). The organism probably follows the oropharyngeal route as its port of entry into the lungs and intestines of both humans and animals (Gardon et al., 2001; Woldehiwet, 2004). It is highly infectious, and a very low dose is sufficient to initiate infection (McQuiston et al., 2002). Primary multiplication takes place in the regional lymph nodes after the initial entry, and a transient bacteraemia develops which persists for five to seven days, as shown in sheep (Woldehiwet, 2004). *C. burnetii* enters monocytes or macrophages; the only known target cells, by phagocytosis in humans (Marrie et al., 1996; Mege et al., 1997). The phagocytic process differs for phase I and phase II bacteria. Phase II bacteria enter the host’s cells through CR3-receptor mediated phagocytosis by activating the CR3 receptors (Mege et al., 1997). On the other hand, the attachment of phase I *C. burnetii* to a monocyte is aided by leukocyte response integrin (LRI) αvβ3, and integrin-associated protein (IAP) (Mege et al., 1997). In spontaneous infections, the phase I *C. burnetii* survives within the phagocytic cells, as the internalisation of the bacteria by these cells is poor. In contrast, uptake of the phase II *C. burnetii* by monocyte is rapid (Mege et al., 1997). After internalisation in phase II, *C. burnetii* initiates phagolysosomal pathways within macrophages which rapidly kill the bacteria (Maurin and Raoult, 1999).

Following passive entry, invading *C. burnetii* bacteria embed in the phagosomes of host cells. Infected phagosomes fuse progressively with lysosomes to form a large vacuole, PV (Hackstadt and Williams, 1981; Howe et al., 2003). *C. burnetii* is an acidophilic bacterium, and therefore, can survive and multiply in an acidic
environment within the PV. Acidic pH allows the entry of necessary nutrients for bacterial metabolism (Chen et al., 1990; Hackstadt and Williams, 1983). It also alters the activity of antibiotics and prevents bacterial killing (Hackstadt and Williams, 1981). Within the PV, C. burnetii undergoes a complex intracellular life cycle to form a spore-like stage. SCVs, the extracellular metabolically inactive and highly resistant form of the bacteria, attach to the host’s cell membrane. After the formation of PV, the acidic environment triggers the activation of the SCVs to form LCVs. LCVs then undergo a sporulation-like process to form a resistant, spore-like form of the bacterium (McCaul and Williams, 1981). Binary fission is the process of cell division for both activated SCVs and LCVs.

C. burnetii is capable of producing chronic infection in both humans and animals, which may persist for months and perhaps, even for years (Marmion et al., 2005). Although C. burnetii persists for a long time within the host cell, it has a very slow intracellular multiplication. Therefore, it may not alter the host cell’s characteristics significantly (Maurin and Raoult, 1999). Moreover, the septum positions itself asymmetrically close to one end of the mother LCV cell during the cell division process to develop a sporulation-like form. So, only one daughter cell receives the PV when an infected cell divides (Hechemy et al., 1993). This phenomenon might also play a role in the persistence of the C. burnetii infection. C. burnetii bacteria show a special affinity to reproductive tissues in pregnant animals. Experimentally, the bacteria have been shown to first infect the trophoblastic cells of chorioallantoic membranes in pregnant goats, and maximum bacterial proliferation was observed in the foetal placental part (Sanchez et al., 2006). This feature might explain the association of C. burnetii infection with abortion.

Both cell-mediated and humoral immunities are required for the clearance of intracellular pathogens (Casadevall and Pirofski, 2006).

In the case of C. burnetii infection in animals, cell-mediated immune response is the key for bacterial elimination (Turco et al., 1984); and the role of humoral immunity is not clearly understood. Two cytokines, namely interferon γ (INF-γ) and tumour necrosis factor (TNF) play the key role in the C. burnetii elimination process (Dellacasagrande et al., 1999; Turco et al., 1984). An in-vitro study has shown that INF-γ can restrict the growth of C. burnetii in infected monocytes (Turco et al., 1984). This cytokine, together with TNF induces an apoptosis of C. burnetii infected monocytes (Dellacasagrande et al., 1999; Mege et al., 1997). It is believed that INF-γ mediates the killing of C. burnetii through the alteration of conditions within the phagosomes of infected cells (Ghigo et al., 2002). Phase I C. burnetii stimulates the synthesis of TNF from the infected monocytes in humans (Capo et al., 1996). It is a general belief that antibodies have little impact on C. burnetii infection (Norlander, 2000). However, during the bacteraemic phase of acute infection, antibodies can play a role in bacterial elimination. But, in chronic infection, immune complexes may propagate pathology (Raoult, 1990). Infection with phase II C. burnetii induces secretion of both IgG and IgM, whereas phase I C. burnetii can only induce IgM production (Maurin and Raoult, 1999).

**Distribution**

Until the recent outbreaks of Q fever in the Netherlands, C. burnetii infections in animals had gained very limited attention, and it was considered to be an infection with very little impact on the health and production of domestic animals. Therefore, information on geographical distribution of animal coxiellosis was very limited. In a recent review article, it was stated that since 1960, C. burnetii infection in domestic ruminants has been reported in 35 countries across five continents (Guatteo et al., 2011). In recent years, Denmark reported an increasing trend of C. burnetii infection (Agger et al, 2014). Human Q fever has been reported from almost all parts of the world, which includes...
all continents except Antarctica (Marrie, 1990; Woldehiwet, 2004). Since 1956, human Q fever has been reported from over 70 countries (Woldehiwet, 2004). The only country that claims to be free from Q fever is New-Zealand. However, a study found evidence of seropositivity in humans (three positive out of 97 serum sample tested) in New-Zealand (Greenslade et al., 2003). Epidemiological studies in man and animals suggested that C. burnetii infection is highly prevalent in tropical regions (Woldehiwet, 2004).

Transmission

C. burnetii bacteria have unique properties that contribute to their transmission between hosts: (1) unlike other members of the Rickettsiaceae, the life cycle of C. burnetii is not dependent on arthropods as vectors; and (2) the SCV form is highly resistant in harsh environment. Inhalation of contaminated fomites is the most common mode of transmission to humans (Marrie, 1990). Domestic ruminants serve as the primary source of human infection (Maurin and Raoult, 1999). However; many other animal species may play a role in C. burnetii transmission. Parturient cattle, ewes and goats can excrete very high quantities of bacteria through amniotic fluid and foetal membranes (Arricau-Bouvery et al., 2005). Direct contamination by aerosols may occur from these products of parturition. Abortive animals may continue to shed bacteria for a long period (Guatteo et al., 2006). Infected animals may also shed C. burnetii in milk, urine, faeces and uterine discharge (Arricau-Bouvery et al., 2005; Guatteo et al., 2006). Milk is the most common shedding route for goats and cattle, whereas ewes shed bacteria most commonly in faeces and vaginal mucus (Rodolakis et al., 2007). Excreted bacteria contaminate fomites such as wool, clothing, straw, manure etc., which may serve as vehicles for transmission (Tissot-Dupont et al., 1999). Indirect transmission to humans may result from the handling of contaminated farm utensils, straw or manure, or by dust from farm vehicles. C. burnetii may also spread through the air, and therefore, infection may occur in a person without any history of animal contact (Marrie and Raoult, 1997; Tissot-Dupont et al., 1999). However, in some studies, it was shown that wind spread is not an important mode of C. burnetii transmission (Gardon et al., 2001). Ingestion of contaminated milk and milk products could be a potential source of human infection (Babudieri, 1959). However, it was not evident in an experimental study (Cerf and Condron, 2006). Rare, but sporadic cases of human-to-human transmission of Q fever have occurred to attendants during autopsies and following contact with a pregnant woman (Raoult and Stein, 1994). Sexual transmission of C. burnetii infection was also reported in a study (Milazzo et al., 2001). Inhalation of bacteria from the infected environment and ingestion of contaminated straw, hay or pasture are likely the most important sources of C. burnetii infection in animals. Animals which live in or come in contact with contaminated premises or infected animals may acquire the infection. Sandford et al. (1994) described three newly purchased goats with a known history of C. burnetii infection introducing infection and abortion in a goat farm. Dogs and wild carnivores may be infected by ingestion of contaminated ruminant placenta or birth products, or by the aerosol route (Angelakis and Raoult, 2010). Although ticks are not essential for the life cycle of C. burnetii, they may still play an important role in transmission of the infection in wild vertebrates (Lang, 1990). Transmission of C. burnetii infection to guinea pigs via tick bites was shown in an experimental study. Possible sexual transmission of C. burnetii infection was reported in mice (Kruszewska and Tylewskawierzbanowska, 1993). Insemination might be a source of infection, as viable C. burnetii was found in bull semen (Kruszewska and Tylewskawierzbanowska, 1997).
**Reservoir**

*C. burnetii* is considered a pathogen with no host specificity and it was shown that infection may occur in a wide range of vertebrates, including wild and domestic mammals, birds and arthropods (Angelakis and Raoult, 2010, Babudieri, 1959, Maurin and Raoult, 1999). Babudieri (1959) stated that *C. burnetii* was detected in virtually all of the animal kingdom. However, the clinical Q fever, is mostly seen in humans. Cattle, sheep and goats are considered to be the most common source of human infection (Marrie, 1990). *C. burnetii* infection, without showing any clinical signs has been reported in horses, pigs, dogs, cats, camels, buffalos, and wild and domestic chickens, pigeons, ducks, geese and turkeys (Babudieri, 1959). This organism was also isolated from rabbits, cats, squirrels, mice, deer and many other free living animals (Marrie et al., 1986). *C. burnetii* was also isolated from 40 species of ticks (Maurin and Raoult, 1999).

**Risk factors**

**Agent factors**

The severity of the infection depends on the strains of the infecting bacteria. Phase I type bacteria are more virulent than the phase II type (Amano and Williams, 1984). Acute infection in humans is caused by *C. burnetii* genomic type I-III, whereas type IV and V are responsible for chronic infection. The virulence of type VI is unknown (Hendrix et al., 1991). Clinical manifestations of Q fever are sometimes dependent upon the route of invasion. Pneumonic lesions are often observed when the infection is gained by inhalation. On the other hand, an infection through the oral route may produce hepatitis (Marrie et al., 1996).

**Host factors**

Age and gender are the two risk factors which are shown to influence the occurrence of Q fever in humans. People aged 30-60 years are the most vulnerable group, and the clinical disease is mostly prevalent in men (Maurin and Raoult, 1999). However, other studies did not find any relationship between gender and the occurrence of Q fever (Marrie and Pollak, 1995). People with a previous history of valvulopathy, an immunosuppressive disease like AIDS and pregnant women (Raoul, 1990; Raoul and Marrie, 1995; Raoul and Stein, 1994) are more prone to develop chronic Q fever. People in certain occupations like veterinarians, animal farm workers, abattoir workers and laboratory personnel are at a higher risk of being infected or seropositive than others; and studies show a comparatively higher prevalence in these groups (Bosnjak et al., 2010, Maurin and Raoult, 1999).

A relationship of *C. burnetii* infection with age and sex was also found in animals, particularly in cattle. Several studies have shown that the prevalence of *C. burnetii* infection increases with age or with the number of parity in cattle and sheep (Bottcher et al., 2011; Garcia-Ispierto et al., 2011; Kennerman et al., 2010; McCaughey et al., 2010, Paul et al., 2014). Prevalence is higher in dairy cows than in beef cattle (McCaughey et al., 2010; Ryan et al., 2011). Among the dairy cattle breeds, prevalence was reported to be higher in Holstein (McCaughey et al., 2010).

**Season, environment and management factors**

Seasonal variation is observed in the occurrence of human Q fever. This variation, however, varies according to geographical region. But most cases of Q fever have been reported in the spring or early summer (Maurin and Raoult, 1999). Human Q fever has been shown to have a relationship with rainfall rather than season (Gardon et al., 2001). A high prevalence of Q fever was observed among people living in close proximity to infected animals or in areas with a high livestock density (Maurin and Raoult, 1999; Smit et al., 2012).

Seasonality in the occurrence of *C. burnetii* infection was also reported in animals. In Japan, most of the Q fever cases in animals were reported in winter (Hellenbrand et al., 2001). On the other hand, in Germany most of the animal cases were reported in
summer (Hellenbrand et al., 2001) and in autumn in Cyprus (Cantas et al., 2011). Increasing animal density increases the infection load in the environment, and is therefore, a potential risk factor of *C. burnetii* infection. Several studies in cattle show that seroprevalence increases with an increasing herd size (McCaughey et al., 2010; Paul et al., 2012). Flock size is reported to have a similar effect in sheep (Kennerman et al., 2010). Several management factors such as housing systems, isolation of a newly introduced animal etc., may also contribute to the seroprevalence of *C. burnetii* infection in animals (Capuano et al., 2001; Paul et al., 2012).

**Clinical manifestation in humans and animals**

*C. burnetii* infections produce both acute and chronic forms of clinical manifestations in humans. However, 60% infection remains asymptomatic with a few patients developing severe illness (Arricau-Bouvery and Rodolakis, 2005; Maurin and Raoult, 1999). The incubation period of Q fever is 2-3 weeks, depending on the route of infection (Maurin and Raoult, 1999). Clinical signs of acute Q fever are nonspecific and vary among patients. A self-limiting febrile condition is the most frequent manifestation in clinical cases, which is accompanied by severe headaches, myalgia, arthralgia and a cough (Tissot-Dupont and Raoult, 2007). A prolonged fever, which may reach 39-40°C, usually stays for 2-4 days and then gradually decreases to a normal level through the following 5-14 days (Maurin and Raoult, 1999). Atypical pneumonia is another common symptom of acute Q fever. Pneumonia is mild in most cases being characterised by a dry cough, fever, and minimal respiratory distress. Patients may also develop hepatitis with hepatomegaly, but without jaundice, subclinical hepatitis and granulomatous hepatitis with a prolonged fever (Maurin and Raoult, 1999). Generally, hepatitis develops in young immunosuppressed patients, whilst pneumonia is often seen in older patients (Fournier et al., 1998). Myocarditis is found in 2% of patients with the acute illness, which may be accompanied by pericarditis (Fournier et al., 2001). Skin rashes and neurologic disorders such as meningoencephalitis or encephalitis, lymphocytic meningitis and peripheral neuropathy have also been observed in acute Q fever cases (Bernit et al., 2002; Maurin and Raoult, 1999). Spontaneous abortion, intrauterine foetal death, premature delivery or retarded intrauterine growth may occur in women that become infected during pregnancy (Carcopino et al., 2007). Pregnant woman may become chronically infected and abort in subsequent pregnancies (Maurin and Raoult, 1999). However, recent studies in Denmark did not find any evidence of association between *C. burnetii* antibody positivity and spontaneous abortion, preterm birth or other adverse pregnancy outcomes (Nielsen et al., 2013). Mortality is a rare outcome of the acute form of the disease. However, severe respiratory distress and myocarditis may lead to death (Fournier et al., 2001).

An infection which lasts for more than six months after the onset is defined as chronic Q fever. This happens in less than 5% of cases (Raoult and Marrie, 1995). The major clinical manifestation of this form of the disease is endocarditis (Tissot-Dupont and Raoult, 2007). It occurs in 60-70% of all chronic cases (Maurin and Raoult, 1999). The case fatality of Q fever endocarditis is less than 10% when patients are treated with antibiotics (Maurin and Raoult, 1999). The aortic and mitral valves are usually affected (Maurin and Raoult, 1999). Unspecific signs like intermittent fever, cardiac failure, weakness, fatigue, weight loss or anorexia may be present. Other manifestations are osteomyelitis, osteoarthritis, chronic hepatitis, hepatomegaly, splenomegaly, digital clubbing, purpuric rash and an arterial embolism (Maurin and Raoult, 1999; Tissot-Dupont and Raoult, 2007). The term coxiellosis is considered to be more appropriate than animal Q fever, as
most cases of animal infection are asymptomatic (Lang, 1990). The organism is found in the blood, lungs, liver and spleen during acute experimental infection, whereas chronically infected animals persistently shed bacteria in their faeces and urine. Infection in most domestic animals remains unrecognised. Coxiellosis is considered a cause of abortion and reproductive disorders in domestic animals (Angelakis and Raoult, 2010). There is scientific evidence to support the hypothesis that C. burnetii can induce epidemics of reproductive failure in sheep and goats, but not in cattle (Agerholm, 2013). Reproductive disorders in domestic animals include endometritis, metritis, stillbirth, reduced birth weight and infertility. In a number of studies, C. burnetii bacteria or antibodies were identified from clinical cases of stillbirth, retained placenta, infertility, endometritis and metritis; however, the evidence was lacking for their association with C. burnetii in cattle (Agerholm, 2013). The herd level perinatal mortality and rate of still birth were not associated with the level of C. burnetii antibodies in bulk tank milk in Danish dairy cattle (Nielsen et al., 2011). If abortions occurred, then the rates generally remained low; however, it ranged from 3-80% (Marrie, 2007). Abortion rate is comparatively higher in ewes and goats than in cows. Abortion is usually observed in late pregnancy in both ewes and cattle (Lang, 1990). In most abortive cases, the aborted foetus appears normal. Discoloured exudate and intracotyledonal fibrous thickening may be observed in an infected placenta. Severe myometrial inflammation and metritis are the frequently observed clinical manifestations in goats and cows, respectively (Arricau-Bouvery and Rodolakis, 2005).

**Diagnosis**

There are no specific clinical signs of C. burnetii infection in humans and animals. Therefore, laboratory diagnosis is a reliable approach to confirm the disease. Since C. burnetii is highly infectious, biosafety level 3 laboratories and experienced laboratory personnel are required to handle the contaminated specimens (Fournier et al., 1998). Several human specimens have to be taken in order to detect C. burnetii. However, the clinical condition of the patient determines the type of specimen. Aborted placenta and amniotic fluid are the best specimens for detection and identification of C. burnetii in female animals. Milk, faeces, urine and uterine discharge can also be used to identify shedder animals. Nowadays, milk and blood samples are widely used to identify seropositive animals.

**Culture and animal inoculation**

Standard biological media are not suitable for the growth of C. burnetii (Maurin and Raoult, 1999). Therefore, isolation of bacteria is done using the shell-vial cell culture techniques or culture in the yolk sacs of embryonated eggs. Guinea pigs, although rarely used nowadays, develop a fever 5-8 days after intraperitoneal inoculation (Woldehiwet, 2004). Further isolation can be done by culturing bacteria in the cell lines or yolk sac from the heavily infected spleen of the inoculated guinea pig.

**Staining and immunodetection**

Fixed impression or smears prepared from aborted placenta, uterine discharge and other secretions from cows, ewes and goats can be stained with Machiavello or modified Ziehl-Neelsen stains to detect the organisms (Angelakis and Raoult, 2010; Woldehiwet, 2004). Antigen detection from impression smears or histological sections can be done by direct or indirect immunofluorescence or immunohistochemical techniques (Woldehiwet and Aitken, 1993), or by fluorescence in situ hybridization (Hansen et al., 2011).

**Serology**

**Complement fixation test (CFT)**

A Complement fixation test (CFT) was a widely used laboratory technique for diagnosis of human Q fever. It is still an OIE recommended test for C. burnetii
infection in animals. The advantage of CFT is that it does not require host specie-specific antibodies (Peter et al., 1985). This test is highly specific, but weakly sensitive (Kovacova et al., 1998; Peter et al., 1985). Moreover, CFT cannot detect early stages of infection as the complement fixing antibodies do not appear in exposed individuals in early stages of the infection (Peter et al., 1985). Therefore, samples from both convalescent and acute phases are required to accurately diagnose the infection. It has been shown that the antigens used in CFT often fail to identify seropositive sheep and goats (Kovacova et al., 1998).

**Indirect immunofluorescence assay (IFA)**

Indirect immunofluorescence assay (IFA) has been widely used and remains a frequently used method for diagnosis of human infection (Bosnjak et al., 2010, Maurin and Raoult, 1999; Nielsen et al., 2013, Tissot-Dupont et al., 1994). It can detect IgG, IgM and IgA against phase I and phase II antigens; and therefore, acute and chronic infections can be differentiated by an IFA test (Tissot-Dupont et al., 1994). In an acute case, antibodies can be detected 7-15 days after the onset of clinical signs. An elevated level of IgG against phase II antigens (≥ 200) and a much lower level of IgM against phase I antigens (≥ 50) indicate an acute infection (Tissot-Dupont et al., 1994). A high level of IgG against both phases I and II and a slightly elevated level of IgA against phase I antigens, although not exclusively predictive, can be detected in a chronic infection. An IgG titre ≥ 800 and an IgA titre ≥ 50 against phase I antigens are often found in chronic infections (Tissot-Dupont et al., 1994). IFA is a species specific test and is not often used for diagnosis of *C. burnetii* infection in animals.

**Enzyme-linked immunosorbent assay (ELISA)**

Several studies in humans have shown that enzyme-linked immunosorbent assay (ELISA) has a higher sensitivity than CFT and IFA (Paul et al., 2013; Peter et al., 1985; Peter et al., 1987). It is recommended as a useful diagnostic tool for sero-epidemiological studies (Peter et al., 1987). ELISA can detect antibodies against both phase I and phase II antibodies (Maurin and Raoult, 1999). This test has a higher sensitivity than the CFT in animal studies (Emery et al., 2012; Horigan et al., 2011; Rousset et al., 2010), and is a quick diagnostic technique (Rousset et al., 2010). It allows the testing of a large number of samples at the same time and is a popular tool for sero-epidemiological studies in animals (Rousset et al., 2010).

**Polymerase chain reaction (PCR)**

Recently, several polymerase chain reaction (PCR) techniques have been developed and successfully used to detect *C. burnetii* DNA in cell cultures and in clinical samples (Berri et al., 2000; Brennan and Samuel, 2003; Fenollar et al., 2004; Fenollar and Raoult, 2007). This technique is highly sensitive and specific, and is a rapid tool for *C. burnetii* detection (Rousset et al., 2010). PCR also has improved the diagnosis of Q fever in veterinary science (Berri et al., 2003). Insertion sequence IS1111 is commonly used reporter and used for primer design in real time PCR for detection and quantification of bacterial DNA (Angen et al., 2011). Superoxide dismutase (sodB) gene; com1 encoding a 27 kDa outer membrane protein; heat shock operon encoding two heat shock proteins (htpA and htpB); isocitrate dehydrogenase (icd); and macrophage infectivity potentiator protein (cbmip) are also detected by PCR.

**Treatment**

Doxycycline at 200 mg daily for 14 days is the recommended regimen for acute cases of Q fever (Maurin and Raoult, 1999). Alternatively, fluoroquinolones can also be used for the patients with meningoencephalitis. Cotrimoxazole and rifampin are also drugs of choice for patients allergic to or in whom tetracyclines contradicted (Maurin and Raoult, 1999). Long-term (>5 weeks) use of cotrimoxazole with folinic acid is recommended for pregnant women (Carcopino et al., 2007). It
has been shown that this treatment is protective against placental infection, obstetric complications and foetal death. Doxycycline (100 mg/day) and hydroxychloroquine (600 mg) are used for the treatment of chronic infections (Carcopino et al., 2007). This treatment, however, must be continued for more than 18 months. Two injections of oxytetracycline (20 mg per kg body weight) in the last trimester of pregnancy are usually recommended for animals, although this may not completely suppress abortions or stop bacterial shedding during parturitions (Berri et al., 2007).

Options for prevention and control
Like other zoonoses, control of Q fever in humans is largely dependent upon the control of C. burnetii infection spread in animals. The control of infection in domestic animals requires knowledge of factors contributing to the introduction and spread of the infection. However, only a few risk factor studies have been conducted. Based on the available knowledge and experience from the recent outbreaks in the Netherlands, it can be concluded that legislative measures on mandatory culling of animals, banding movement and transport, mandatory vaccination are important tools for controlling Q fever (Roset et al. 2011b). Improved biosecurity measures (e.g., avoiding contact between farm animals and visitors, quarantine of newly introduced animals and improved hygienic precautions of farm personnel) may also play roles in controlling C. burnetii infection (Agger et al., 2013; Paul et al., 2012). Other control measures to reduce the number of infected animals and environmental contamination are: i) addition of lime or calcium cyanide at 0.4% for treatment of manure (Angelakis and Raoult, 2010); ii) safe disposal of aborted foetuses, foetal membranes and contaminated bedding materials (Woldehiwet, 2004); and iii) thorough cleaning and disinfection of utensils and transporting vehicles (Woldehiwet, 2004). Appropriate measures should be taken to reduce the tick burden in the environment. Biosecurity measures such as avoiding contact between farm animals and visitors, quarantine of newly introduced animals and improved hygienic precautions of farm personnel should be implemented in animal farms and industries (Woldehiwet, 2004). Avoiding consumption of infected raw milk and pasteurisation of milk and milk products can contribute to reduction of Q fever burden in humans (Woldehiwet, 2004).

In the recent outbreaks in some EU Member States, a wide range of control measures were pursued in order to minimise the exposure to humans and to reduce spill-over from animals to humans. These included the restriction of infected farm visits in the Netherlands (EFSA, 2010; Roset et al. 2011b; Van den Brom and Vellema, 2009), avoiding human gatherings in high-risk areas (EFSA,2010; Panaiotov et al., 2009); the closing of schools during an outbreak during 2004 in Bulgaria (Panaiotov et al., 2009); banning blood donation programs in affected areas in France during 2002 and 2007, and in Germany during 2005 (EFSA,2010;Georgiev et al., 2013; INVS, 2009); shifting infected herds/flocks from human locations in Bulgaria during 2004 (Panaiotov et al., 2009); and introduction of ban on animal movements (Georgiev et al., 2013). Furthermore, changes in the farming practices including manure management such as covering and natural composting or ploughing of manure, treating manure with lime or calcium cyanide have been implemented in the Netherland, Germany and France (EFSA,2010;Roset et al. 2011b; Van den Brom and Vellema, 2009, Vellema et al. 2010). Removal of animal birth and abortion products (EFSA, 2010; Georgiev et al., 2013), disinfection of infected premises in Bulgaria during 2004 (Panaiotov et al., 2009); and implementation of a farm animal breeding ban in the Netherlands during 2007–2010 (EFSA, 2010; Roset et al. 2011b), have also been practised. Moreover, in 2010, more than 50,000 pregnant small
ruminants from PCR bulk tank milk positive farms were culled in the Netherlands with an aim of reducing the shedding of *C. burnetii* in order to reduce the human exposure from environmental contamination. This culling program was supplemented by a program of repopulation with immunised animals from PCR bulk tank milk negative farms only. A compensation scheme for the farmers affected by the culling program was instituted (Roest et al., 2011a; Roest et al., 2011b). However, the effectiveness of different control measures remains uncertain. It has been reported that the prevalence of *C. burnetii* in an infected herd usually declines over time, even without taking any control measures. This is probably due to the ‘natural’ immunisation of susceptible animals (Georgiev et al., 2013). However, enough strong scientific evidence is still unavailable to make a final conclusion.

Q fever is considered to be a professional hazard, therefore, vaccination is primarily considered for those who are professionally at risk such as livestock farmers, producers of animal products, veterinarians and laboratory workers (Maurin and Raoult, 1999). Three types of vaccines can be used for preventing Q fever in humans: the attenuated live vaccine, a chloroform-methanol residue extracted vaccine and other extracted vaccines and the whole-cell formalin-inactivated vaccine. A vaccination program for people at risk from chronic Q fever, such as patients with cardiac valve disease, aortic aneurisms and vascular prostheses was launched in the Netherlands in July 2010, and commenced in January 2011, after the Q fever outbreak had subsided (Georgiev et al., 2013; van der Hoek, 2012). Vaccinations have been shown to reduce abortion, shedding of *C. burnetii* and the occurrence of infection in animals (Arricau-Bouvery and Rodolakis, 2005; Hogerwerf et al., 2011; Kovacova and Kazar, 2002). It has been recommended that vaccination be sustained for several consecutive years (Astobiza et al., 2011; de Cremoux et al., 2012). Outbreak vaccination, i.e. vaccinating herds that are already infected (Astobiza et al., 2011; Guatteo et al., 2008) or otherwise under high infection pressure (de Cremoux et al., 2012), is less effective than regular vaccinations. Phase I vaccine is recommended for animals, as it is more protective than the phase II vaccine. The vaccination of animals was implemented during the outbreak in France during 2009; and in the Netherlands during 2007-2010 (Hermans et al., 2011; Rodolakis, 2009; Roest et al., 2011b). In the Netherlands, the vaccination program was initiated in October 2008, through a voluntary scheme involving dairy sheep and dairy goats on farms with more than 50 goats or sheep, petting zoos and nursing farms in a restricted high risk zone. This program was made mandatory in January 2010, subsequently introduced in an enlarged area (EFSA, 2010; Georgiev et al., 2013; Roest et al., 2011b). Nationwide mandatory vaccination coverage was achieved in 2011, which also included small ruminants attending animal shows (EFSA, 2010).

**CONCLUSION**

*C. burnetii* is a widely distributed bacterium with zoonotic potentials. Since its first discovery in 1937, knowledge about this bacterium and the disease Q fever has increased quite a lot. However, there are still knowledge gaps which require further scientific studies. *C. burnetii* infection in domestic animals has been reported in many countries including Bangladesh. Despite this, very little is known about the pathogenesis of *C. burnetii* infection in humans and domestic animals. It is also known to be a cause of reproductive failure in domestic animals, including cattle. However, available literature has failed to prove the association between infection and the reproductive failures in cattle, and this area demands more systematic studies.
Although there is a long history of existence, the complete epidemiology of *C. burnetii* infection has not been explored sufficiently. Albeit mostly remains asymptomatic, this infection has the potentiality to produce considerable negative impact on both human and animal health, as it have shown in the recent outbreaks in EU countries.

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