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Review

ROLE OF *COXIELLA BURNETII* IN THE DEVELOPMENT OF FEVER OF UNKNOWN ORIGIN: A MINI REVIEW

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Summary

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Q fever is a widespread zoonosis throughout the world in the form of numerous natural and agricultural outbreaks. *C. burnetii* infects various hosts, including humans, ruminants and pets and in rare cases, reptiles, birds, and ticks. This bacterium is excreted in urine, milk, faeces, and birth products. In humans Q fever occurs as acute or chronic disease with diverse clinical presentation, as isolated cases and epidemics. It affects various organs and systems, and in pregnant women can cause miscarriage or premature birth. Untreated Q fever can become chronic with adverse effects on patients. Diversity in the clinical picture in the absence of specific pathological syndrome often hinders accurate diagnosis and proper etiological significance. Therefore, improvement of diagnostic methods and in particular the development and introduction of new molecular diagnostic methods is the basis of effective therapeutic and prophylactic approach. The purpose of the review is to renew the interest to Q fever – on one hand, because of its serious impact on human health and agricultural systems, and on the other, the ability for development and introduction of new molecular diagnostic methods.

Key words: *Coxiella burnetii*, clinical manifestation, diagnostics, fever of unknown origin, therapy

INTRODUCTION

Q fever is an infectious zoonosis, caused by *Coxiella burnetii* – an obligatory intracellular bacterium. It is widespread, which is considered a public health problem in many countries (Süss *et al*., 2004). It is estimated that so far only New Zealand is not affected (Hilbink *et al*., 1993). *C. burnetii* infects various hosts, including

humans, ruminants (cattle, sheep, goats), pets and, in rare cases, reptiles, birds, and ticks (Angelakis & Raoult, 2010). This bacterium is excreted in urine, milk, faeces and birth products (Cutler *et al*., 2007; Rodolakis, 2009). *C. burnetii* infection in livestock often goes unnoticed. The infection in humans is variable – from asymptomatic till a wide range of acute and chronic manifestations. The clinical signs vary greatly from patient to patient. The most common manifestation of acute Q fever is a self-limited flu-like syndrome accompanied by fever of unknown origin (FUO) and atypical pneumonia (Honarmand, 2012). In rare cases (< 5%), *C. burnetii* infection becomes chronic (Healy *et al*., 2006) with devastating results, especially in patients with pre-existing valvular heart disease. Complications are cirrhosis, hepatitis, encephalitis, endocarditis, pericarditis, myocarditis, interstitial pulmonary fibrosis, and meningitis (Healy *et al*., 2011).

Since the clinical presentation is very pleomorphic and nonspecific, the incidence of Q fever among humans is probably underestimated and diagnosis particularly relies upon the physician's awareness of the symptoms of Q fever and the presence of a reliable diagnostic laboratory. Therefore, the diagnosis must be considered in the case of a FUO, especially if the fever recurred following contact with possibly contaminated mammals. It is beneficial for all physicians because of the wide range and the nonspecific nature of the clinical manifestations and the fact that the diagnosis is posed too late and diseased patients are treated inadequately, especially patients with clinically silent, previously undiagnosed valvulopathies and endocarditis (Million *et al.*, 2013).

HISTORICAL DATA

For the first time, the Q fever was recognised in 1935 and described in 1937 by E. Derrick, when he studied an unknown disease outbreak among abattoir workers in Brisbane, Australia (Derrick, 1937). The predominant symptom was a fever of unknown origin (called "query" – vague, undefined). Two years later H. Cox, G. Davis, F. Burnet and M. Freeman isolated the causative agent of the disease. In Europe, hundreds of cases of Q fever illness were described during the 1940s among soldiers in Greece, Ukraine, Corsica and others (Lipton *et. al.*, 1987).

CLASSIFICATION AND BACTERIOLOGY

Phylogenetic studies classify *C. burnetii* into the γ-subgroup of proteobacteria, Legionellales order and *Coxiellaceae* family, genus *Coxiella*, based on low diversity in gene, which is evolutionarily conservative and encoding 16S rRNA (Weisburg *et al.*, 1989; Stein *et al.*, 1993). There are almost 20 different *Coxiella* genotypes distributed into six groups (group I to group VI), due to variations in the composition of lipopolysaccharide (LPS), that showed a distinct pattern of association with acute and chronic diseases (Van Schaik *et al*., 2013). Interestingly, isolates from group I, II and III are associated with acute infections, whereas groups IV and V consist of isolates associated with chronic infections (Glazunova *et al*., 2005).

Coxiella burnetii is a Gram-negative obligate intracellular pathogen. It expresses pleomorphic forms with dimensions 0.3–0.6 μm (Fournier *et al*., 1998). The main structural elements of *Coxiella* are the cell wall, cytoplasm, nucleoid and surface structures (Chmielewski & Ty-

lewska-Wierzbanowska, 2012). Structures whose size correspond to ribosomes were detected in the cytoplasm. The cell wall contains a peptidoglycan layer, which is composed of N-acetylmuramic acid, N-acetylglucosamine, D-alanine, D-glutamic acid, and meso-diaminopimelic acid. The outer membrane includes a complex of LPS (Amano & Williams, 1984). *C. burnetii* demonstrates antigenic variation of LPS chemical composition, similar to that observed in the *Enterobacteriaceae* family. These variations determine the form of the bacterial cells in phase I or phase II. Phase I is the natural form, occurring in infected animals and humans, characterised by high infectivity. Phase II is not very infectious and occurs in laboratory conditions (Amano & Williams, 1984). Lipopolysaccharides are the only antigen and immunogen, which differ in phase I and II of *C. burnetii*. This antigenic specificity is crucial for serological differentiation between acute and chronic Q fever (Hackstadt *et al*., 1985; Ordi-Ros *et al.,* 1994).

C. burnetii possesses a small chromosome, 5 Mbp in size, and one to five plasmids of 30–51 kbp, containing about 2% of the genetic information. So far, 2134 gene sequences, involved in adhesion, invasion, intracellular trafficking, host modulation and other related functions have been identified. Among all detected gene sequences, 719 (33.7%) have no homologues of gene sequences known in other bacteria. These hypothetical genes may be responsible for functions important in the unique developmental cycle of *C. burnetii* (Chmielewski & Tylewska-Wierzbanowska, 2012).

EPIDEMIOLOGY

Q fever is a zooantroponosis, considered as public health problem in many countries in Europe, America, Japan, Israel and Australia. There are many environmental reservoirs, including wild, farm animals, pets and arthropods, mainly ticks (Fournier *et al*., 1998). Although in wild animals the main route of infection is blood sucking by ticks, in domesticated and farm animals it is realised by inhalation of aerosolised organisms (Sawyer *et al*., 1987; Lang, 1990). *Coxiella* is excreted in faeces, urine, and milk and especially through the amniotic fluid and placenta (Muskens *et al*., 2007; Guatteo *et al.*, 2011). In animals the infection is generally asymptomatic, but abortions, stillbirths and reproductive disorders in ruminants may occur. The infected animals may shed the agent for more than 4 months.

Ticks are not only vectors, but reservoirs of infection. It is estimated that more than 70 species of ticks are carriers of *C. burnetii*. Main vectors are ticks from *Ixodes*, *Rhipicephalus*, *Amblyomma* and *Dermacentor* genera. Infection with them is life-long. Transovarial and transphasic transmission of *C. burnetii* has been shown (Sprong *et al*., 2012). Bacteria are able to penetrate and multiply in epithelial cells of the intestine and in the midgut from where are excreted with faeces and contaminate animal haircoat (Maurin & Raoult, 1999).

Humans are infected with *C. burnetii* via inhalation of contaminated aerosols, by alimentary route and rarely by tick-borne transmission. A very low infective dose is sufficient for infection. Therefore, Q fever is an occupational hazard. At greatest risk are persons in contact with farm animals, but also laboratory personnel working with infected animals (Johnson & Kadull, 1966; Angelakis & Raoult, 2010). The oral route of infection is less important (Salmon *et al*., 1982; Fishbein

& Raoult, 1992) due to the higher resistance of the intestinal mucosa compared to the respiratory epithelium (Fournier *et al.*, 1998; Reusken *et al*., 2011). Transmission among humans through sexual contacts has also been described (Kruszewska *et al*., 1996; Millazo *et al*., 2001). Such infections are reported during autopsy from necropsy material, blood transfusion (Raoult & Marrie, 1995), contact with infected parturient woman (Maurin & Raoult, 1999), bone marrow transplantation (Loudon & Thompson, 1988; Leski *et al.*, 2011) and in immunocompromised patients, including AIDS (Heard *et al*., 1985). *C. burnetii* may persist asymptomatically in humans throughout life. However, pregnancy, a cardiac valvular abnormality, a vascular aneurysm or prosthesis, haemodialysis (Leonetti *et al*., 1995), and immunodeficiency, including AIDS (Loudon & Thompson, 1988), may promote reactivation of dormant *C. burnetii*. All age groups are susceptible to *C. burnetii*, these in active working age 45–65 being more affected with to clinical disease. Infected men prevail over women (Maurin & Raoult, 1999). In Europe, acute Q fever cases are more frequently reported in spring and early summer (Raoult *et al.,* 2005). Q fever is usually benign, but mortality occurs in 1 to 11% of patients with chronic Q fever (Raoult, 1990; Kampschreur *et al*., 2012). For example, since 2007 the largest Q-fever epidemic ever reported in the Netherlands has been lasting until nowadays. During 2007–2010 over 4,000 human cases have been recorded, with 14 deaths. The main reason of the epidemic is the increase in the intensity of goat breeding and ineffective animal vaccination (Hogema *et al*., 2012; Tilburg *et al*., 2012). Q fever is the leading rickettsial disease in Bulgaria. For more than 50 years numerous sporadic cases, small and large epidemics, ranging from several tens to hundreds of ill people are documented. These were the outbreaks in 1984–1985 in the regions of Knezha and Pavlikeni, in Panagyurishte in 1993 with 1269 registred ill patients (Alexandrov *et al.,* 1994; Martinov, 2007). In 2002 and 2004 there were two major outbreaks in the areas of Etropole and Blagoevgrad, which 200–300 affected patients, including children (Martinov, 2007). In Bulgaria, no vaccination was used against Q fever, therefore this disease is widespread. Permanent control over natural and agricultural outbreaks is essential in predicting morbidity, likelihood of outbreaks and timely prevention.

CLINICAL MANIFESTATION

Since the clinical presentation is very pleomorphic and nonspecific, the incidence of Q fever among humans is probably underestimated and diagnosis particularly relies upon the physician's awareness of the symptoms of Q fever and the presence of a reliable diagnostic laboratory. The latter is beneficial for all physicians because of the wide range and the nonspecific nature of the clinical manifestations and the fact that the diagnoses registered in recent years, outbreaks of Q fever are made too late and patients are treated inadequately.

Q fever shows a wide range of acute and chronic manifestations. The incubation time is 1–3 weeks. About 60% of infected persons are asymptomatic. The others have a non-specific influenza-like illness with fever, headache, sweats, dry cough, myalgias and arthralgias in acute phase of infection. Most of them develop atypical pneumonia confirmed by chest radiography, and/or hepatitis. In patients with hepatitis, the liver biopsy reveals

diffuse granulomatous changes and blood testing shows high levels of alkaline phosphatase and transaminases (Honarmand, 2012; Maurin & Raoult, 1999). Hospitalised patients are about 2 percent of the infected ones.

Acute Q fever

Fever of unknown origin (FUO). In patients with acute Q fever, the flu-like form of the disease is the commonest clinical manifestation. The temperature may rise to 39–40 °C. In most cases the febrile response is accompanied by headache. In untreated patients temperature period may be extended, which is a factor for diagnosis of FUO. Temperature has a biphasic nature. The first phase corresponds to that described above. In the second, body temperature is lower of 1 to 9 days duration. In older patients febrile illness lasts longer and in 28% of cases fever relapsed (Derrick, 1937; Cutler *et al*., 2007;).

For the last three years 618 clinical specimens from 532 patients with clinical diagnosis of FUO have been submitted for testing in the National Laboratory of Rickettsiae and Tissue Cultures. By indirect qualitative enzyme immunoassay (ELISA) analysis and one-step PCR 67 patients with acute Q fever were confirmed. Reactive *C. burnetii* IgM antibodies in combination with headache, fever, fatigue, arthralgia etc. were the commonest clinical signs in these patients. In some cases the musculoskeletal system, cardiovascular and nervous system were affected. The decisive factors, defining the clinical manifestation were the route of infection, the different infective dose and the characteristics of the infecting strains (Pavlova, 2016).

Pneumonia. The pulmonary form (pneumorickettsiosis) is the second most commonly encountered clinical manifestation. In the majority of patients with acute pneumonia caused by *C. burnetii*, fever, chills, headache and diaphoresis are established. Usually there is no cough, even when pneumonia is detected radiologically. Signs detected with a lower frequency in patients are nausea, vomiting, pain behind the sternum, diarrhoea, and sore throat. The course of the disease is favourable and moderate. About 55% of patients with pneumonia caused by *C. burnetii* have splenomegaly and hepatomegaly. Lethality is less than 3%, most often in patients with concomitant diseases of the lungs and heart (Marrie, 1985). Clinical and laboratory changes most often occur with leukopenia, left shift, eosinopenia and thrombocytopenia, leukocytosis in severe cases. Erythrocyte sedimentation rate is moderately increased. Laboratory findings are nonspecific: anemia, thrombocytopenia, leukocytosis, hypergamma globulinaemia. Findings from chest radiography are nonspecific. The duration of symptoms varies from 10 to 90 days. The mortality rate ranges from 0.5 to 1.5%, depending upon the series (Derrick, 1937; Tissot-Dupont *et al*., 1992; Honarmand, 2012).

Hepatitis. In acute Q fever, regardless of the clinical form, in most cases there is liver damage. Hepatitis is settled mainly laboratory, in 70% transaminase levels are increased 2–3 times above the norm. In rare cases progressive icterus and hepatomegaly are present. There are also reports of acute liver failure and hepatic coma (Marrie, 1988; Rice *et al*., 1993; Dupont *et al.*, 1994).

Myocarditis. Myocarditis is a rare but life-threatening clinical manifestation. It occurs in 0.5 up to 1% of etiologically proven cases (Raoult *et al.*, 1986; 1999).

Other manifestations. As late onset of acute Q fever are described individual

cases of encephalitis, meningoencephalitis and encephalomyelitis (Drancourt *et al*., 1991; Marrie & Raoult, 1992). Rarely, haemolytic anemia, thyroiditis, pancreatitis, epididymitis, Guillain-Barre syndrome, and mediastinal lymphadenopathy have been also described (Fournier *et al*., 1998).

Skin changes in patients with Q fever are nonspecific. Macular or bright red papules located on the body, which rarely becomes petechial have beenfound in 5 to 21% of patients. (Marrie, 1985; Dupont *et al*., 1994; Kazar, 1996).

Chronic Q fever

Unlike acute, chronic Q fever is a serious and often fatal disease. Its mortality ranges from 10% to 60% depending on diagnosis and treatment. It occurs in 5% of cases from 1 year to 20 years after infection with *C. burnetii*. Typically, the heart is the most commonly involved organ, followed by arteries, bones, and liver (Brouqui *et al*., 1993). Endocarditis is the main clinical manifestation of chronic Q fever and constitutes 60–70% of all cases. It occurs when burdened patients with cardiac valvular and vascular damage who underwent transplantation and/or immunosuppression from cancer, chronic renal failure and is associated with the ability of *C. burnetii* to multiply in histiocytes of connective tissue (Raoult *et al*., 1993; Honarmand, 2012). Most often, the mitral and aortic valve are affected. Symptoms are non-specific. Arterial embolism occurs in about 20% of patients (Stein & Raoult, 1995). Vegetations are only rarely seen by transthoracic cardiac ultrasonography (Jortner *et al.* 1994). Because of the lack of specificity of symptoms, the diagnosis is often delayed with 12 to 24 months, resulting in an increased mortality rate.

Other, less specific events, are moderate to febrile temperature, sweating, fatigue, weakness, chills, rash, hepato/splenomegaly, icterus, embolism, etc. (Raoult *et al.*, 1990; Brouqui *et al*., 1993). Key role in the diagnosis of Q fever endocarditis has the valvular disease combined with unexplained inflammatory syndrome. The clinical signs are non-specific and similar to those of other endocarditis types, which explains why diagnosis is delayed for months, even years. With adequate therapy, lethality can be reduced to 10%.

Q fever in children

Q fever is uncommon in children. The main clinical symptoms are pneumonia with pleural effusion, meningitis, rarely hepatitis (with elevated transaminases) and haemolytic-uremic syndrome. Fever is ascertained in all infected children and lasts for 10 days. About half of affected children complain from headache, abdominal pain, nausea, and fatigue (Maltezou *et al*., 2004). In childhood chronic Q fever occurs as endocarditis and/or recurrent osteomyelitis and osteoarticular infections (Cottalorda *et al*., 1995). Cases of clinically manifested Q fever in children in Bulgaria have been observed during the Panagyurishte epidemic in 1993 (Mitov *et al*., 2008).

Q fever during pregnancy

There are a lot of data on the consequences of Q fever during pregnancy. In untreated women it is associated with high foetal mortality. Normal outcome and a healthy child at delivery were present in only 18.9% of monitored untreated women. Infection during pregnancy is associated with higher risk of developing chronic form of the disease and spontaneous abortions in the future. Q fever can also be activated during the next preg-

nancy, and therefore patients require regular monitoring and long-term cotrimoxazole therapy (Marrie, 1993; Ludlam *et al*., 1997; Stein & Raoult, 1998).

SPECIFIC LABORATORY DIAGNOSIS OF Q FEVER

Coxiella burnetii can be demonstrated in various ways, depending on the type of sample and the purpose of investigations (Samuel & Hendrix, 2009; Sidi-Boumedine *et al.*, 2010). Diagnosis of Q fever relies on cultural, microscopical, serological and molecular methods. Blood cultures are typically negative. For diagnosis, different samples are delivered in laboratory, i.e. ticks, whole blood, plasma, surgically removed heart valves, prosthetic valves, aneurysms, vascular grafts, organ samples from biopsies, placentas and aborted fetuses, vaginal discharges. For investigation of bacterial shedding, samples can betaken from vagina, milk, colostrum and faeces (Kim *et al*., 2005; Guatto *et al*., 2007; 2008).

Methods, used for investigation and diagnosis of *Coxiella burnetii* are presented in Table 1.

Cultural methods

As an obligate intracellular bacterium, *Coxiella burnetii* can be isolated by inoculation of specimens into conventional cell cultures (Raoult *et al*., 1990; 1994), embryonated chicken yolk sacs (Ormsbee, 1952) or laboratory animals (Williams *et al.*, 1986). Inoculation of laboratory animals (guinea-pig, mouse, hamster) is helpful in cases requiring isolation from tissues, faeces, milk or environmental samples contaminated with various microorganisms. *Coxiella* is classified as biosafety level 3.

Microscopic methods

C. burnetii can be seen on smears or frozen tissue prepared with a routine Giemsa stain (Gimenez, 1964). Because it is acid resistant, the bacteria can be stained by several methods: Stamp, modified Ziehl-Neelsen, Gimenez, Giemsa and modified Koster. Because of lack of specificity, a positive finding is presumptive evidence of Q fever.

Serological methods

Among the various techniques that can be employed, the three most often used are: the indirect immunofluorescence assay (IFA), the ELISA and the complement fixation test (CFT). Three older serological tests are no longer used in routine diagnosis: the microagglutination technique, the capillary agglutination test and the indirect haemolysis test (proposed for chronic Q fever) (Doller *et al.*, 1984). Until relatively recently, the CFT and the micro agglutination assay were the methods employed in the detection of antibodies against *C. burnetii*. CFT detects complement-fixing antibodies I and II phase present in the serum. The CFT is specific, but less sensitive than the ELISA or IFA (Kittelberger *et al.,* 2009; Rousset *et al.,* 2007). Sometimes, the so called zone phenomenon that leads to false positive results is observed. Micro agglutination assay requires a large amount of antigen (Nguyen *et al.*, 1996).

In human medicine, the IFA adapted as a micro-immunofluorescence technique is the current method for serodiagnosis of Q fever (Tissot-Dupont *et al.,* 1994). It is now considered the reference method or "gold standard" for the diagnosis of Q fever by serology. It has proved to be the most sensitive and specific way for detection of *Coxiella* antibodies. The procedure can be adapted to perform an immunopero

Table 1. Methods of investigation and diagnosis of *Coxiella burnetii*

Methods	Description
Cultural methods	Requires BSL-3 level of biosafety. Relatively slow. Isolation of *C. burnetii* is made on cell cultures, on 6–7 day-old chick embryos (Ormsbee, 1952) and laboratory animals (Williams *et al.*, 1986).
Microscopic methods	
Staining	Gimenez, Giemsa-Romanowski and other methods.
Immunodetection	Immunofluorescence and immunoperoxidase methods are applicable in the early days of the disease (Jensen *et al.*, 2007)
Serological methods	
Indirect immunofluorescence	Reference method for serodiagnostics of Q fever (Peacock *et al.*, 1983). The required amount of antigen is very small. It determines antibodies from phase I and II.
Complement fixation (CFT)	Less specific and sensitive method than IF (Kittelberger *et al.,* 2009; Rousset *et al.,* 2007). Detects I and II phase antibodies.
Microagglutination	A simple and sensitive method that can detect early antibody response to *C. burnetii*. Requires a large amount of antigen (Nguyen *et al.*, 1996).
Microimmunofluoresence method (MIF)	Reference serological test for Q fever (Tissot-Dupont *et al.,* 1994).
Enzyme immunoassay (ELISA)	More specific and more sensitive than CF. Proposed as a good method for seroepidemiological studies (Peter *et al.*, 1987).
Immunoblot test	Specific and sensitive method for the diagnosis of Q fever (Blondeau *et al.*, 1990), but is time-consuming.
Dot immunoblot assay (dot immunoblotting)	Equally specific and sensitive as ELISA and MIF test, but more specific than CF (Cowley *et al.*, 1992).
Indirect haemolytic test	Proposed for chronic Q fever (Doller *et al.*, 1984).
Radioimmunological test	Based on use of radioactively labelled ^{125}I (Doller *et al.*, 1984).
Molecular techniques	
DNA amplification	PCR is used successfully for detection of *C. burnetii* DNA in cell cultures and clinical specimens (Stein & Raoult, 1992a,b).
Genotyping methods	MLVA and MST are very useful for epidemiological investigation, particularly to clarify links regarding source of infection (Arricau-Bouvery *et al.,* 2006; Svraka *et al.,* 2006).

xidase assay. Based on detection of phase I and II antibodies (between acute and convalescent paired sera); a 4–fold rise in complement-fixing antibody titre against phase II antigen occurs and yields the highest specificity. Typically, titres of ≥1:800 are considered positive for IgG in chronic Q fever (Fournier *et al.*, 1996) and titres of ≥ 1:50 for IgM and ≥ 1:200 for IgG (Tissot-Dupont *et al.,* 1994). As usual, seroconversion or titre levels that increase 4-fold are regarded as positive for the detection of an acute or reactivated infection.

Enzyme-linked immunosorbent assay (ELISA) has a high sensitivity and a good

specificity (Kittelberger *et al.,* 2009; Rousset *et al.,* 2007) and is proposed as a good method for seroepidemiological studies (Peter *et al.*, 1987). It is easy to perform in laboratories that have the necessary equipment (a spectrophotometer) and reagents. The ELISA is preferred to IFA and CFT. Ready-to-use kits are commercially available and can detect anti-phase II antibodies or both anti-phase I and II antibodies. False-positive serologic results may occur in legionellosis and leptospirosis.

Antigen detection methods

Detection of *C. burnetii* in samples can also be achieved by specific immunodetection (immunohistochemistry), *in-situ* hybridisation or DNA amplification (Jensen *et al.*, 2007; Samuel & Hendrix, 2009).

Fluorescence *in-situ* hybridisation using specific oligonucleotide probes targeting 16s rRNA may be used on paraffin embedded tissues, especially placenta samples (Jensen *et al.,* 2007). No specific antibodies for immunochemistry are commercially available.

PCR methods have been used successfully to detect *C. burnetii* DNA in cell cultures and biological samples, such as serum, resected cardiac valves, etc., with greater sensitivity than serum assays. The PCR methods of *C. burnetii* detection are generally performed for the health investigations of ruminant herds or flocks prone to abortions (Sidi-Boumedine *et al.*, 2010). *Coxiella* can be detected in the blood by PCR 2 weeks after transmission. This technique can be performed in suitably equipped laboratories using primers derived from various targets such as: superoxide dismutase (sodB) gene, encoding 27 kDa outer membrane protein (Stein & Raoult, 1992a,b), heat shock operon, encoding two heat shock proteins (htpA and htpB), isocitrate dehydrogenase (Klee *et al*., 2006) and macrophage infectivity potentiator protein.

The real-time PCR provides an additional means of detection and quantification (Kim *et al.,* 2005; Klee *et al.,* 2006;). Various target genes are used (Klee *et al*., 2006; Sidi-Boumedine *et al.*, 2010). To quantify the bacteria in biological samples using the real-time PCR, it is recommended to amplify a unique and specific sequence. Different primers and probes used in PCR can be obtained from http://ifr48.timone.univmrs.fr/Fiches/Fievre_Q.html#toc22, regularly updated by the French Reference National Center for human Q fever.

Genotyping methods

Several typing methods have been used for the characterisation of *C. burnetii* strains, such as restriction endonuclease of genomic DNA (Hendrix *et al.,* 1991), PFGE (pulsed-field gel electrophoresis) (Jager *et al.,* 1998), and sequence and/or PCR-RFLP (restriction fragment length polymorphism) analysis. More recently, two PCR-based typing methods have been described, MLVA (multi-locus variable number of tandem repeats analysis) (Arricau-Bouvery *et al.,* 2006; Svraka *et al.,* 2006) and multispacer sequence typing (MST) (Glazunova *et al.,* 2005) that permit the typing of *C. burnetii.* MLVA and MST are considered to be the most discriminating methods for *C. burnetii*, allowing the identification of up to 36 distinct genotypes. Moreover, databases have been established for MLVA (http://minisatellites.u-psud.fr/MLVAnet/) and MST (http://ifr48.timone.univ-mrs.fr). These tools are very useful in epidemiological investigations to clarify links regarding source of infection, for better understanding the epidemiological emerging factors.

TREATMENT OF Q FEVER

Treatment of acute Q fever should be started within the first 3 days of disease. The drugs of choice are tetracycline (4×500 mg), primarily doxycycline (2× 100 mg), administered for 14 days. An alternative for tetracyclines are fluoroquinolones (3×200 mg) for 14–21 days, or pefloxacin (400 mg) administered 14–20 days (Raoult, 1993; Maurin & Raoult, 1999). Effect was also observed after macrolides application. Clinical studies have shown that erythromycin administered at doses of 500 mg every 6 hours for 10 days, is effective as well. Acute forms of Q fever during pregnancy are treated with rifampicin or trimethoprim/sulfamethoxazole, known to be relatively well tolerated during pregnancy at the usual therapeutic doses. If progress is associated with distinct signs of inflammatory activity a seven day course of prednizone (40 mg/24 h) is parallelly conducted. Chronic Q fever (myocarditis) requires a long treatment with tetracyclines, for months or even years. Despite that, treatment failure or relapse of the disease often occur (Ellis *et al.,* 1983).

CONCLUSIONS

Even described many years ago, Q fever is still a poorly understood disease. Due to the variability in the clinical signs often it remains unrecognised, thus the number of cases of Q fever is likely underestimated. The diagnosis Q fever must be considered in all cases of fever of unknown origin, especially in people from risk groups, working with or being in contact with potentially infected animals. The important role of farm animals, especially ruminants in Q fever epidemiology, requires a closest collaboration between veterinary and medical specialists in this field.

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