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Coxiella burnetii

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1. Introduction

Q fever is a zoonosis caused by *Coxiella burnetii*, a small obligate intracellular Gram-negative pathogen worldwide spread, except New Zealand (Maurin & Raoult, 1999).

Q fever has been described for the first time in 1935 as an outbreak of fever in workers of a slaughterhouse in Brisbane, Australia (Derrick, 1937). Derrick could not identify the aetiological agent therefore he defined the disease as “query fever”. Afterwards, in 1937, Burnet and Freeman (Burnet & Freeman, 1937) isolated a troublesome intracellular pathogen from mice inoculated with blood or feces from Derrick’s patients, and classified it as *Rickettsia burnetii*. Only in 1948 Philip (Philip, 1948), according to cultural and biochemical characteristics, re-classified *R. burnetii* as a new genus, *Coxiella*, in honour of Herald R. Cox, who first isolated this microorganism in the USA.

Ruminants and pets represent the most important reservoirs of the infection, and transmission to man mainly occurs through inhalation of contaminated aerosols. The disease is characterized by a wide clinical spectrum, varying from asymptomatic seroconversion, self-limiting febrile episodes to hepatitis and pneumonia. The illness can also occur in a chronic form, mainly characterized by endocarditis, and sometimes can have a lethal outcome. In contrast in animals *C. burnetii* infection is generally asymptomatic, even if infected animals can shed intermittently this pathogen in feces, urine, milk and birth products. Clinical symptoms eventually occurring in ruminant herds are mainly represented by reproductive disorders, such as premature birth, dead or weak offspring and infertility.

2. Etiology

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Phylogenetic and molecular studies based on the 16S rRNA sequences locate *C. burnetii* in the order of Legionellales, in the gamma group of Proteobacteria, next to bacteria such as *Legionella* spp., *Francisella tularensis* and *Rickettsiella* spp. (Raoult et al., 2005). In eukaryotic cells *Coxiella* replicates inside vacuoles, in mammals prefers monocytes and macrophages. This pathogen exhibits a complex intracellular cycle, characterized by the formation of spore-like forms. In infected cells it can be found in two different forms, one metabolically inactive, denominated SCV (“Small-Cell Variant”) and the other one metabolically active, LCV (“Large-Cell Variant”). The SCV form appears as a small and compact rod, and is highly resistant to physicochemical agents, such as desiccation and common disinfectants, therefore exhibiting high persistence in the environment. The LCV form is instead bigger and less dense if observed at the electronic microscope; it is metabolically active and can

differentiate in the SCV form through a sporogenic differentiation. Following cell rupture, the spore-like forms are released in the external environment where they can survive for long periods.

When cultured on embrionated eggs or cell cultures, *C. burnetii* exhibits an antigenic variation associated with loss of virulence. Indeed this microorganism shows a pathogenic form denominated phase I, isolated from animal or human infected cells, and an avirulent form, denominated phase II, isolated after serial passages on embrionated eggs or cell cultures. The attenuated phase II is characterized by a deletion in the chromosome which causes the loss of some cell surface determinants (Maurin & Raoult, 1999).

The association between specific characteristics of *C. burnetii* and the virulence of the strain is still an open controversial question, and several theories have been proposed to address the ability of different isolates to induce the acute or the chronic form of the disease. Several researchers tried to correlate the virulence to specific phenotypic and genetic profiles of the microorganism. In particular, SDS-PAGE analysis of the LPS isolated from the phase I allowed to identify three phenotypic groups antigenically distinct, associated, the first one, with acute episodes of the infection in different sources (ticks, bovine milk and man), the second and the third one with chronic episodes in man (Hackstadt, 1986). Studies in literature show that *Coxiella* possesses four different plasmids, QpH1, QpRS, QpDV and QpDG7 (Valková & Kazár, 1995). According to the harboured plasmid and also to DNA restriction profiles, *C. burnetii* can be divided in six genetic groups, variably correlated to specific pathotypes (Hendrix et al., 1991). In particular, the groups from I to III, carrying the plasmid QpH1, have been isolated from ticks, human cases of acute Q fever, bovine milk and birth products of domestic ruminants; the groups IV and V, that respectively possess the plasmid QpRS or any plasmid (but, in the latter case, they possess plasmidic sequences integrated in the chromosomal DNA), have been associated with abortions of domestic ruminants and chronic human cases of endocarditis or hepatitis; the group VI, carrying the plasmid QpDG, isolated from rodents, results avirulent in experimental mouse models of the infection (Stoenner et al., 1959; Stoenner & Lackman, 1960). Subsequent studies classified 80 *C. burnetii* isolates in 20 different genetic profiles by RFLP analysis (analysis of the Restriction Fragments Length Polymorphisms). According to this characterization, four profiles correspond to the former genetic groups I, IV, V and VI, respectively. In general, the RFLPs profiles seem to be associated with the geographical origin of the analysed isolates (Jäger et al., 1998). More recent studies based on MST (Multispacer Sequence Typing) analysis have variedly confirmed the existence of the aforesaid genetic groups, and identified three great monophyletic groups, containing the groups I, II and III, the group IV and the group V, respectively. Moreover, these studies seem to confirm the association between the plasmid QpDV and acute infections, and between the plasmid QpRS and chronic infections, and between specific genotypes and specific courses of the disease (Glazunova et al., 2005). Subsequent studies (Beare et al., 2006) performed by microarray analysis, further confirm the RFLP genetic groups, and add to the previous groups from I to VI, other two big groups (VII and VIII). These studies, underline the importance of the polymorphisms of the genes involved in LPS biosynthesis for the virulence of *C. burnetii*. An opposite school of thought instead, considers the characteristics of the host as the main cause of the course of the infection in relation to the development of acute or chronic diseases. These theories follow on the fact that isolates carrying the plasmid QpH1 have

been isolated in France from both acute and chronic human Q fever episodes, while isolates deprived of the plasmid QpH1 have been shown to be able to induce an acute syndrome as well (Stein & Raoult, 1993). Models of the disease have therefore been proposed in which the same isolate is able to cause either the chronic or the acute form of Q fever, exclusively according to the host immune response. Particularly, the establishment of the chronic syndrome has been associated with a compromised immune state of the host, as for instance in case of HIV infection, as well as with an increased production of IL-10, responsible of a diminution of the ability of the macrophages to eliminate *C. burnetii* due to an inhibitory effect on the phagosome maturation process (Raoult et al., 2005). Recent data (Russell-Lodrigue et al., 2009) on the behaviour of *C. burnetii* isolates belonging to the genetic groups I, IV, V and VI in mouse models of Q fever seem however to confirm the theory of the association genotype-pathotype. In this study, in fact, strains associated with mice acute episodes (group I) have been shown to cause a faster progression of the disease, to induce greater pathological changes and to exhibit a higher speed of proliferation *in vivo* if compared to isolates collected from chronic episodes (groups IV and V). Moreover, the isolates of the group I, if compared to the others, induce in mouse a stronger immune response, characterized by a greater production of inflammatory cytokines for a longer period of time.

3. Sources of infection and routes of transmission

Sources of infection of Q fever are diverse, and the principal for man is represented by the inhalation of infected particles (Fig. 1). Transmission of the bacterium through contaminated aerosol can occur following direct contact with infected animals, mostly, with birth products, such as amniotic fluid and placenta, which can in turn contaminate the new born or the skin of other animals. The extreme resistance of this pathogen to external agents makes it persistent in the environment, especially in areas where domestic ruminant farms are present. In fact *C. burnetii* can be easily transmissible through contaminated hay or contaminated dust, and spread in the surrounding environment by the wind. For this reason cases of Q fever can also be recovered in patients that have not had evident contacts with animals. Human-to-human transmission results extremely rare, although cases of transmission of Q fever occurred through contact with parturient, through the transplacental way (congenital infection), through sexual relations (shown in mouse models of the infection) (Kruszewska & Tylewska-Wierzbanska, 1997), blood transfusions (van der Hoek et al., 2010) and intradermic inoculation. Ticks are considered as natural reservoirs of *C. burnetii*. They contribute to the maintenance of the infectious agent in the environment by transmitting the disease to animals (livestock, pets and wildlife) by bite or by expelling heavy loads of *C. burnetii* with their feces which can contaminate the skin of animals or be inhaled (Kazar, 1996). Cats, dogs, rabbits, foxes and rodents are thought to constitute a reservoir for maintenance of infection in the domestic cycle of Q fever (Aitken, 1989). The role of rats has not yet been clearly described, even if recent data suggest that they might be true reservoirs of the infection, capable of independent maintenance of *C. burnetii* infection cycles thereby contributing to spread and transmission of the pathogen (Reusken et al., 2011).

C. burnetii is secreted in the milk, therefore the ingestion of contaminated food such as raw milk and dairy products, represent a possible source of infection for humans (Maurin & Raoult, 1999). Hirai and colleagues analysed 147 cheese samples by PCR analysis and found

19% of positive results (Hirai et al., 2011). However, when inoculated in mice, none of the positive samples allowed the recover of viable *C. burnetii*. Also, the administration of contaminated milk to voluntaries provided contradictory results (Angelakis & Raoult, 2010). The notable transmissibility of *C. burnetii* makes this microorganism extremely dangerous, particularly for occupationally exposed workers (veterinarians, slaughterhouse workers and farmers) and for laboratory technicians in contact with potentially contaminated specimens which therefore require manipulation by experienced personnel in BL3 facilities (Angelakis & Raoult, 2010).

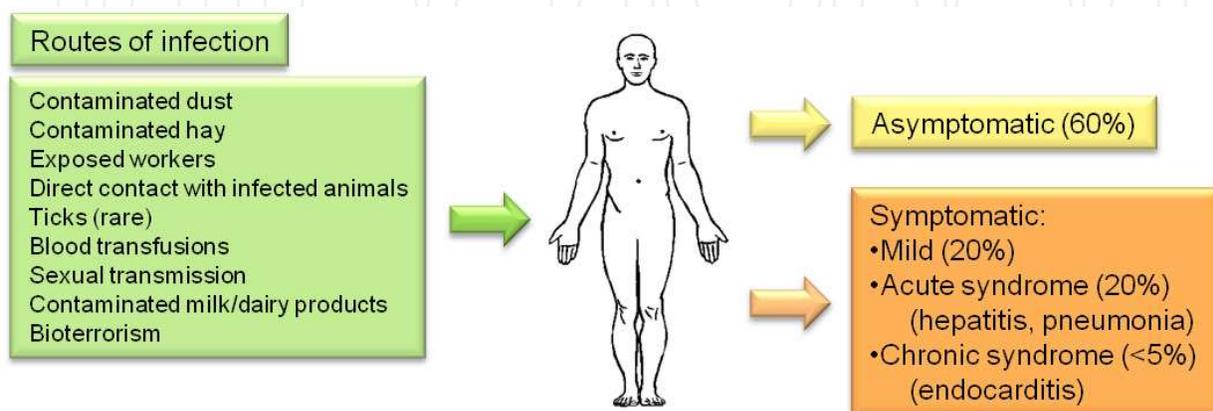


Fig. 1. Routes of transmission and pathophysiology of Q fever

C. burnetii is considered as a potential bioweapon, and is classified as class B agent (Madariaga et al., 2003). The high transmissibility of this pathogen, its extreme ability to persist in the environment and the aerial route of transmission make it a suitable bacterial agent for bioterrorism actions. In fact it has been evaluated that the inhalation of only one bacterial cell would be enough to induce the disease in man; moreover, the World Health Organization has esteemed that if 50 kg of *C. burnetii* were spread as an aerosol in an urban area of 500.000 inhabitants, 125.000 cases of acute illness, 9.000 cases of chronic Q fever and 150 dead people would be recorded (World Health Organization, 2004).

The aerial route of transmission of Q fever also represents the principal mode of infection for animals, for which however, unlike humans, an important role in the spread of the disease is also played by ticks. Other important ways of infection for animals are represented by direct contact with infected animals in the herd, by ingestion of contaminated placentas or milk, as well as possible ingestion of infected wild rodents (Angelakis & Raoult, 2010).

4. Pathogenesis

C. burnetii is a strictly intracellular bacterium capable of infecting different cellular types, mainly monocytes and macrophages. Its entry in the host cell is allowed by a mechanism of phagocytosis, much more efficient towards the phase II, avirulent, than to the phase I, virulent. This difference is due to the fact that the attachment to phase I bacteria is exclusively mediated by the integrin $\alpha v \beta 3$, while attachment to phase II bacteria is mediated both by the integrin $\alpha v \beta 3$ and by the complement receptor CR3. The most efficient

internalization process determines a best intracellular replication and explains why bacteria in phase II grow faster than those in phase I, therefore justifying the conversion of the phase I to the phase II following growth in cell cultures. Both types can be recovered in the phagosomes, but only the phase I can survive in macrophages, while the phase II is quickly eliminated. The ability of *C. burnetii* to grow inside eukaryotic cells is due to the adaptation to the intracellular acidic pH. A pH value of 4.5, indeed, allows the entry of nutrients necessary for the metabolic functions of the bacterium, and, at the same time, confers protection from the action of numerous antibiotics, by altering their bactericidal activity. In macrophages this microorganism locates in vacuoles where its survival and proliferation are achieved by the control of phagocytosis and the prevention of phagosome lysosome fusion (Angelakis & Raoult, 2010; Raoult et al., 2005). The incomplete maturation of the phagosome is due both to the loss of the expression of the cellular marker cathepsin D, and to the exogenous production of IL-10, that also interferes with the microbicide activity of the macrophages. Interferon- γ restores the fusion between the phagosome and the lysosome, therefore allowing the elimination of *C. burnetii*. Moreover, it induces the alkalization of the vacuoles and controls the metabolism of the ions in the macrophages, inhibiting therefore the intracellular bacterial replication. Interferon- γ , finally contributes to the elimination of the infected macrophages through apoptosis, by inducing the expression of TNF on the cellular membrane. Following infection, the production of specific immunoglobulins is observed; in particular, while the phase I only stimulates the production of IgM, the phase II stimulates the production of both IgM and IgG1. The acute syndrome of Q fever determines a cell-mediated immune response and the formation of characteristic granulomatous lesions with a classical open space in the middle and a fibrin ring ("doughnut" granulomas). The control of the acute form includes the action of T cells, that however generally results insufficient for the complete elimination of the bacterium (Honstetter et al., 2004). When the infection assumes a chronic form, the level of inflammation becomes elevated, while cell-mediated immunity becomes defective. In fact, it has been observed that in patients affected by chronic endocarditis, the production of the inflammatory cytokines TNF and IL-6 is increased, while the ability of the lymphocytes to proliferate in response to the stimulation with *C. burnetii* antigen is decreased (Koster et al., 1985).

5. Epidemiology

Q fever is a worldwide zoonosis, prevalent in most countries in the world, with the exception of New Zealand. The reservoirs are large but partially known, and include many domestic and wild mammals, birds and arthropods like ticks (Fig. 2). The main sources of infection for man are domestic ruminants, mainly cattle, sheep and goats. Animals are often chronically infected, but mostly asymptomatic. In females *Coxiella* locates in the uterus and in the mammary glands and is shed in the environment through birth products, feces, urine and milk (Babudieri, 1959; Marrie & Raoult, 2002). Also pets, included dogs, cats and rabbits, can transmit the infection to man (Marrie & Raoult, 2002; Stein & Raoult, 1999). Animals can be infected by tick bites, ingestion of infected placentas or milk, and through the inhalation of contaminated particles. In ticks, as in mammals, *C. burnetii* is in phase I, and therefore highly contagious. Ticks, however, are not considered essential in the diffusion of the pathogen to domestic ruminants, but they play an important role in the

transmission of *Coxiella* to the wild fauna, included vertebrates, lagomorphs and birds. Moreover they can spread high quantities of the microorganism through their feces, which in turn can be inhaled both by man and animals. Age and gender seem to have a role in the pathogenesis of Q fever; in particular, studies in man show that subjects less than 15 years old are less sensitive than older subjects (Angelakis & Raoult, 2010). Moreover, the number of cases in men is 2.5 times greater in comparison to that recorded in women (Gikas et al., 2010). During pregnancy, in animals, Q fever becomes chronic and *C. burnetii* remains in the uterus and in the mammary glands with the possibility to be reactivated by following pregnancies (Marrie et al., 1996).

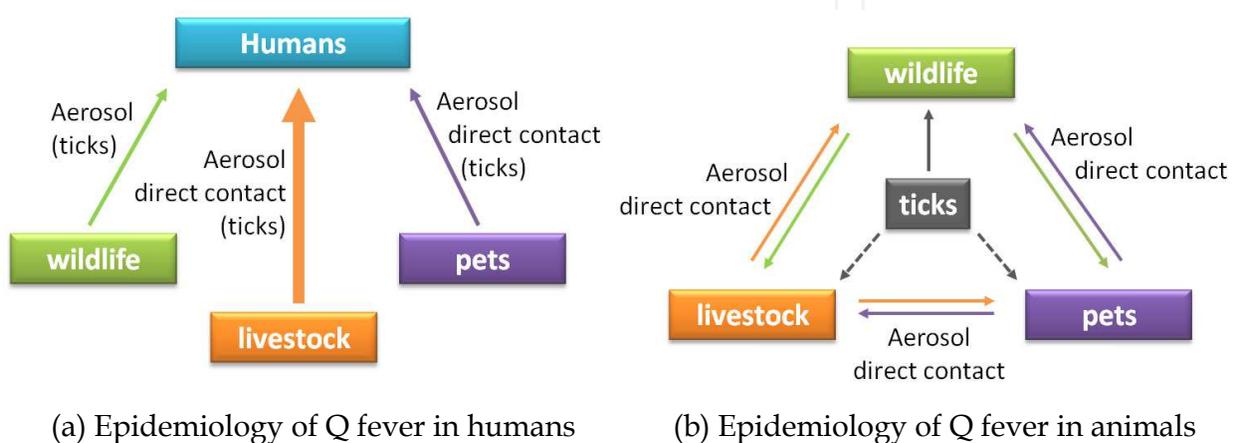


Fig. 2. Epidemiology of Q fever

5.1 Occurrence of Q fever in man

Q fever can appear in the form of sporadic cases or outbreaks. It is a notifiable disease, but, as the infection is often asymptomatic, and its mild forms can be mistaken for other febrile episodes, sporadic forms of the disease are often undiagnosed and the true incidence of Q fever is still unknown. Moreover, the indiscriminate use of antibiotics in febrile patients hampers the clinical identification of Q fever as well as other rickettsioses and bacterioses. To the moment in the European states it is not clear yet the exact entity of Q fever in man, and in domestic ruminants. In Europe, preliminary data point out that during 2007, 585 human cases have been notified, while in 2008 they increased to 1594, with an increase of 172%. High risk groups include people working with possible infected material (slaughterhouse workers, veterinarians, meat-processing workers), persons living in or next to farms, and laboratory personnel processing eventually infected organs and tissues (Borriello et al., 2010; EFSA Panel on Animal Health and Welfare, 2010).

In abattoirs and wool-processing plants several epidemic outbreaks have occurred worldwide. Australia is considered an endemic area, with an outbreak characterized by 2000 cases in 1979-1980 (Hunt et al., 1983), while in Uruguay, in 1976, 310 of 360 workers and veterinary inspection personnel in a meat-packing plant contracted the disease in a month. The outbreak was attributed to the inhalation of contaminated aerosols, probably generated by the handling of infected material, as most cases were recorded among workers involved

in bone-milling or collection of animal wastes. Three more outbreaks occurred in the same meat-packing plant in 1981 and 1984, mainly involving personnel working with slaughtering and animal wastes treatment (Somma-Moreira et al., 1987). Epidemics still occur in other slaughterhouse workers as well. Recent cases have been reported in New South Wales and in Scotland (Gilroy et al., 2001; Wilson et al., 2010).

Several Q fever epidemics have also been reported in farms and in geographic areas close to domestic ruminant herds (cattle, sheep and goats). Q fever cases have also been recorded in scientific institutes working with sheep as study models in different countries (Hall et al., 1982; Meiklejohn et al., 1981), or in human pathology institutes, as reported by Gerth and colleagues (1982) in Germany. Major outbreaks also occurred in the years following the World War II with estimated 20,000 cases in a two years period (Babudieri, 1959). In an urban school in central Israel a large Q fever outbreak has been reported in 2005, possibly transmitted by the air conditioning system (Amitai et al., 2005). This report highlights the importance to investigate the seroprevalence of Q fever in influenza-like outbreaks occurring outside the influenza season.

In addition to cattle, sheep and goats, parturient cats and newborn kittens can also represent a source of infection for man (Marrie et al., 1989). The role of cats in human Q fever epidemiology should not be underevaluated as it is the result of a combination of high prevalence of *C. burnetii* in rats and cats' predatory behavior towards these natural reservoirs (Reusken et al., 2011).

In the USA, 132 cases of Q fever with onset in 2008 have been reported; of these, 117 were acute Q fever and 15 were chronic Q fever (NASPHV & CDC, 2011). A serological survey in Japan reported prevalence values in healthy humans of 22.2% (Htwe et al., 1992). Moreover it has been observed that in Japanese children Q fever is often characterized by a clinical expression, mostly represented by atypical pneumonia (Hirai & To, 1998). A recent outbreak in the Netherlands started in 2007 and made the European Commission concerned about this zoonosis. The Dutch epidemic counted more than 2,200 confirmed human cases of the disease and more than 20 people died. In an effort to prevent the disease from spreading further, over 50,000 dairy goats were slaughtered and the government launched a mandatory animal vaccination campaign at the start of 2009. The main cause of infection has been attributed to infected goat and sheep farms located in the southern Brabant province (EFSA Panel on Animal Health and Welfare, 2010).

All the observed epidemics have common risk factors. Particularly, the greatest part of cases appear associated with a strict contact with domestic ruminant herds/flocks, mainly sheep and goats, both in relationship to the location and to the routes followed by the flocks. A great number of cases has often been recorded during or immediately after the period of the parturitions. Weather conditions characterized by dry and windy climate seem to play an important role in the transmission of the disease. Moreover, another risk factor is represented by living and/or working, sporting or performing social activities near agricultural zones covered with manure. Finally, also the presence of additional natural reservoir of infection (such as wild animals or ticks) near inhabited zones or herds further contributes to the maintenance of the pathogen in the environment (Borriello et al., 2010). The extreme persistence of *C. burnetii* in the environment and its diffusion in domestic

ruminants and wild animals indicate therefore that good management practices play a critical role for the control of Q fever, not only in animals, but also in man (EFSA Panel on Animal Health and Welfare, 2010).

5.2 Occurrence of Q fever in animals

Q fever has been found in almost all species of domestic animals and many wild species. In India, the agent was isolated from amphibians (NASPHV & CDC, 2011) and a python. Q fever is endemic in domestic ruminants in the greatest part of the world. Serological surveys conducted in endemic areas have revealed a sizable proportion of reactors in the bovine, ovine and caprine populations. Although the infection is common, the disease is rare, and it has a limited impact on animal health.

Studies on the seroprevalence of the infection in cattle reported values of 67% in Ontario (Lang, 1989) and 40.4% in Sudan (Reinthal et al., 1988). Similar prevalence values have also been reported in sheep and goats flocks in California (Ruppanner et al., 1982) and in Sudan (Reinthal et al., 1988). In Japan, a survey on the presence of Q fever in domestic ruminants reported values of 25.4% in healthy cattle, 28.1% in sheep and 23.5% in goat. In Japanese bovine herds with reproductive disorders the seroprevalence of Q fever reached values of 84.3% (Htwe et al., 1992). In the European states to the moment it is not clear yet the exact entity of Q fever in domestic ruminants, since rules or recommendations are not harmonized for monitoring and report of the disease (EFSA Panel on Animal Health and Welfare, 2010). Epidemiological data on Q fever in animals point out that the general prevalence of the disease in domestic ruminants is increased from the 7.4% to the 10.0% within the period 2007 -2008. Particularly, the greatest increase has been recorded for goats, with values of 9.7% and 15.7% in 2007 and in 2008, respectively. Member States mostly affected are Bulgaria, France, Germany and, in particular way, the Netherlands (EFSA Panel on Animal Health and Welfare, 2010). In Italy *C. burnetii* is widely spread in domestic ruminants. Molecular analysis of milk in bovine farms has shown a prevalence of the infection of 40% (Magnino et al., 2009), and a significant association has been found between seropositive animals and abortion (Cabassi et al., 2006).

Reservoir species of *C. burnetii* can also be found among wildlife species and arthropods like ticks. A serological and molecular study in the Netherlands carried out on brown and black rats collected from both livestock farms and urban areas found the 15.8% of the brown rats seropositive, and detected *C. burnetii* DNA in the spleen of 4.9% of the brown rats and 3.0% of the black rats by PCR analysis (Reusken et al., 2011). A recent study in Northern Spain (Astobiza et al., 2011) identified as potential sources of the disease several wild species, such as roe deer, wild boar, European hares and birds, by PCR detection of *C. burnetii* DNA in spleen and liver (5.1%, 4.3%, 9.1% and 1.2%, respectively). Another study also carried out in Spain reported higher prevalence values in farmed red deer than in wild red deer (40% vs. 5.6%), probably indicating that in farmed animals direct contact may increase the risk of *C. burnetii* transmission (Ruiz-Fons et al., 2008). Moreover, several findings from different authors show an active role of ticks in maintaining *C. burnetii* in both wild and peridomestic cycles, therefore indirectly representing a risk factor for transmission of Q fever to humans (Mediannikov et al., 2010; Parola & Raoult, 2001; Toledo et al., 2009).

5.3 Occurrence of Q fever in domestic ruminants in Southern Italy

In Southern Italy the presence of *C. burnetii* in bovine and water buffalo herds, and in ovicaprine flocks has been reported (Galiero et al., 1996; Parisi et al., 2006; Perugini et al., 2009), even if the exact prevalence of this pathogen is still largely unknown. The presence of *C. burnetii* in bovine and water buffalo herds of the Campania region was therefore investigated by molecular analysis carried out on aborted fetuses collected during the period 2009-2011.

For this purpose a total of 69 fetuses was analysed. The DNA from several organs (liver, lung, abomasum and placenta) was extracted by the DNA mini kit (QIAGEN) and was subsequently amplified by a single-tube nested PCR for the detection of *C. burnetii* (Parisi et al., 2006). The fetuses were considered positive when at least one of the sampled organs resulted positive.

The obtained results have shown that the 47% (8/17) of the analyzed bovine fetuses and the 29% (15/52) of the analyzed water buffalo fetuses resulted positive to *C. burnetii* PCR detection (Tab. 1).

Species	Examined Foetuses	Positive Foetuses
Bovine	17	8
Water buffalo	52	15
Total	69	23

Table 1. PCR detection of *C. burnetii* in bovine and water buffalo fetuses

Among the 8 positive bovine fetuses, 4 (50%) exhibited the presence of *C. burnetii* in the lungs, 1 (12%) in the liver and 5 (62%) in the abomasum. One foetus exhibited the presence of the pathogen both in lungs and liver.

Among the 15 positive water buffalo fetuses, 8 (53%) exhibited the presence of *C. burnetii* in the liver, 7 (47%) in the lungs, 3 (20%) in the abomasum and 4 (27%) in the placenta. Four fetuses exhibited the presence of *Coxiella* both in liver and lungs, one foetus in liver, lungs and placenta, and another foetus in liver, lungs and abomasum (Tab.2).

Species	Analysed foetuses	Number of positive samples			
		Lungs	Liver	Abomasum	Placenta
Bovine	8	4	1	5	0
Water buffalo	15	7	8	3	4
Total	23	11	9	8	4

Table 2. Detection of *C. burnetii* in organs

Abortions in bovine and water buffalo herds determine serious economic losses. In Southern Italy the incidence of abortions caused by infectious diseases is elevated and clear

epidemiological data able to explain the possible causes of this phenomenon are still lacking (Capuano et al., 2004). Particularly, among the aetiological agents responsible of abortion, preliminary reports indicate that the presence of this pathogen is significant in Southern Italy. Indeed, Parisi and colleagues (2006) reported an incidence of *C. burnetii* in bovine herds and ovi-caprine flocks of 6% and 21.5%, respectively, while, in water buffalo herds, variable inter-herd prevalence values have been found, ranging from 17 to 23% (Galiero et al., 1996; Perugini et al., 2009).

The data reported in this study confirm the presence of *C. burnetii* both in bovine and water buffalo herds, and point out that this pathogen plays an important role as abortive agent for these animal species. Moreover, water buffalo seems to be more susceptible to this pathogen rather than cattle. Other studies will be therefore necessary to clarify the epidemiology and the pathogenesis of *C. burnetii* infection both in cattle and water buffalo. Particularly water buffalo needs careful investigation as the milk from this species can be used also crude for the production of the world famous “mozzarella di bufala” cheese.

The single-tube nested PCR proved to be an efficient diagnostic method to determine the presence of *C. burnetii* in organs from bovine and water buffalo fetuses. This technique, indeed, exhibited enhanced sensitivity, as it is based on the amplification of a secondary target sequence within the first run product. The use of a nested PCR therefore might increase the possibility to detect *C. burnetii* DNA in animal tissues, where DNA can easily be degraded due to autolysis phenomena, as it often happens in animal fetuses.

Moreover, these data underline the need of specific diagnostic methods to be carried out within proper monitoring plans aiming to provide a careful estimate of *C. burnetii* prevalence and its routes of transmission.

6. Description of the disease

Q fever is characterized by a polymorphic clinical spectrum, therefore the diagnosis of the disease can only be made if systematic laboratory tests are performed. Several factors are likely to influence the course of the infection by *C. burnetii*. Among these the route of infection, the infectious dose, age and gender play a major role (Angelakis & Raoult, 2010).

6.1 Q fever in man

In man the clinical demonstration of the disease can consist in an asymptomatic seroconversion, an acute form of the illness, varying from self-limiting fever episodes up to granulomatous hepatitis or severe pneumonia, or a chronic form, characterized by endocarditis. In the acute forms the incubation period has an average duration of 20 days, and the greatest part of cases presents no or mild symptoms. The combination of symptoms varies greatly from person to person, and it can include high fevers (up to 39-40°C), severe headache, general malaise, myalgia, chills and/or sweats, non-productive cough, nausea, vomiting, diarrhoea, abdominal pain and chest pain. Atypical pneumonia is among the most common acute manifestations of *C. burnetii* infection. In some patients serious respiratory stresses and diffusion of the bacterium in the pleura can occur. The duration of symptoms varies from 10 to 90 days, with a mortality of about 0.5-1.5%. Hepatitis is the most common

clinical manifestation of Q fever, and it can be either asymptomatic, or associated with hepatomegaly or with characteristic granulomas and prolonged fever (Angelakis & Raoult, 2010). Rarely (2% of the cases) other symptoms can occur, such as pericarditis, myocarditis, neurological symptoms (varying from the most common headaches to meningitis, meningoencephalitis or peripheral neuropathies). Dermatological lesions are more common than generally thought and they include transient punctiform rashes, maculopapular eruptions and, more rarely, erythema nodosum (Raoult et al., 2005). Post-Q fever fatigue syndrome can be observed in some (10-25%) acute patients, characterized by constant or recurring fatigue, night sweats, severe headaches, photophobia, pain in muscles and joints, mood changes and difficulty sleeping. In pregnant women *Coxiella* settles in the uterus and mammary glands, often resulting in pre-term delivery or miscarriage.

Chronic Q fever is a severe disease occurring in <5% of acutely infected patients. It may present soon (within 6 weeks) after an acute infection, or may manifest years later. The chronic forms are mostly characterized by endocarditis and vascular infections, less frequently by aortic aneurysms and infections of the bone, liver or reproductive organs, such as the testes in males. They almost exclusively affect individuals with predisposing conditions, such as lesions of cardiac valves, vascular problems or immunodeficiency, and symptoms can also occur months or years after the infection. The endocarditis and the vascular infections from chronic Q fever generally have a lethal outcome if they are not treated with an appropriate antibiotic for a period of at least 18 months up to a life treatment (Maurin & Raoult, 1999).

6.2 Q fever in animals

In domestic ruminants *C. burnetii* infection is mostly asymptomatic. During the acute phase *C. burnetii* can be found in blood, lungs, spleen and liver, whereas during chronic Q fever it is persistently shed in urine and feces.

In cattle the main pathological demonstrations of Q fever associated with chronic infections are represented by ipofertility, metritis and low birth weight calves, more rarely by abortions and stillbirths. In asymptomatic but seropositive herds, *Coxiella* is almost exclusively shed in milk. The excretion can last several months (up to 32) and can be continuous or intermittent, and, in some cases, can be associated with chronic subclinical mastitis (Rodolakis et al., 2007). In bovine herds with reproductive disorders, infected females primarily shed *Coxiella* through birth products, but also through feces, urine and milk (Arricau Bouvery et al., 2003). Elimination of the microorganism through these ways can persist for several months, also for subjects that have not exhibited problems during the parturition (Berri et al., 2005b). None of these shedding patterns however appears to have a predominant role in the eliminatory subjects, often exhibiting one excretion route only.

Sheep and goats, like cattle, are considered as the main reservoirs of infection for man. They almost always result asymptomatic and the most common pathological manifestations of chronic Q fever are abortions and stillbirths. In sheep and goats flocks with reproductive disorders, animals contemporarily shed the bacterium through vaginal mucus, feces and milk. Particularly, in a recent work, goats have been shown to eliminate *C. burnetii* mostly through milk, sheep mainly through vaginal mucus or feces (Rodolakis, 2009).

Asymptomatic but seropositive ovine flocks, instead, always resulted negative to *C. burnetii* detection by PCR analysis in bulk tank milk.

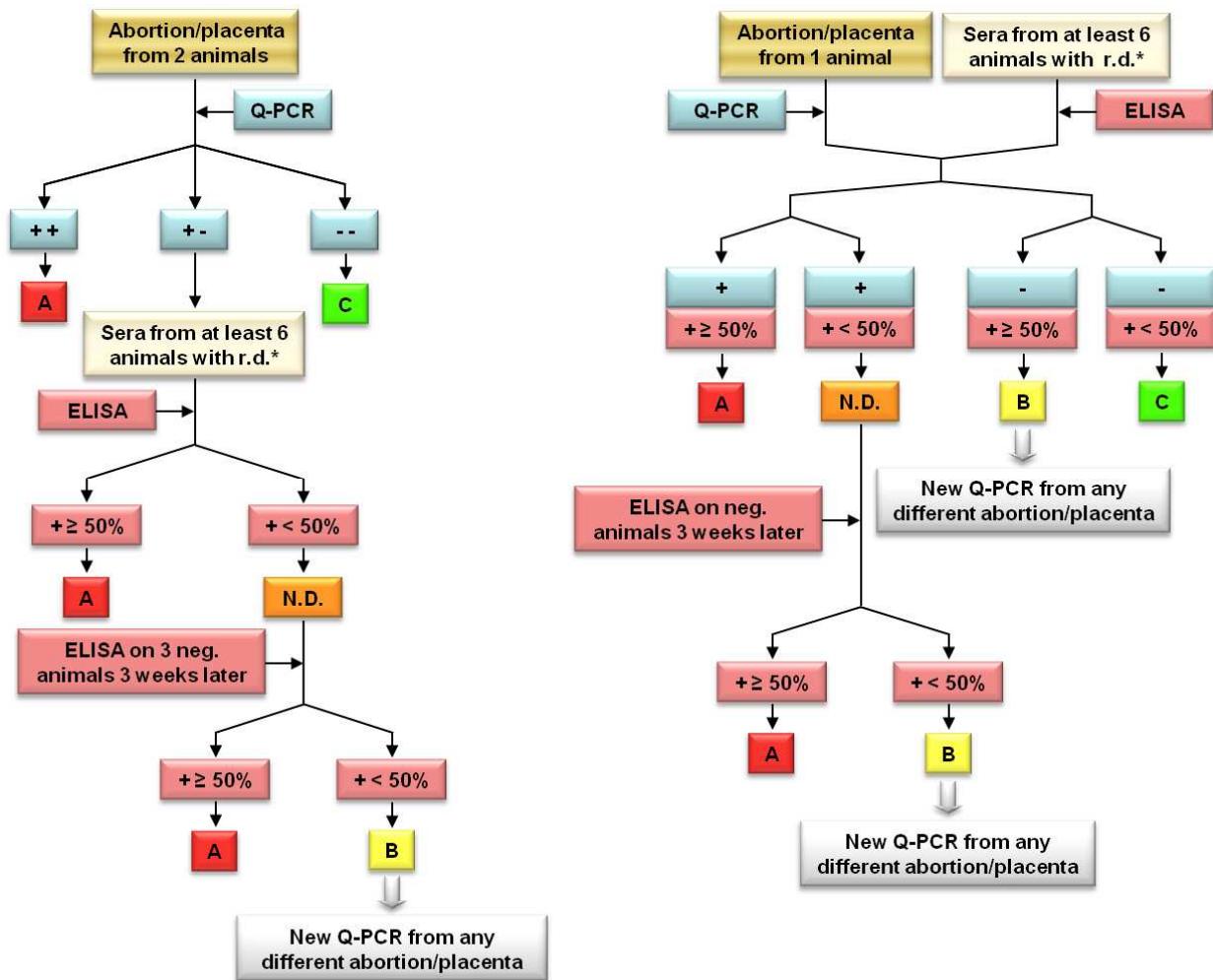
Differences in shedding patterns can explain why sheep and goats are identified more frequently than cattle as the main source of infection for man (Rodolakis, 2009).

7. Diagnostic techniques

The extreme virulence of *C. burnetii* requires the use of bio-containment level 3 facilities for contaminated specimens processing, and isolation and cultivation of the pathogen should be performed by experienced laboratory personnel only (OIE, 2010). *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).

For human diagnosis of Q fever the most appropriate tests are PCR analysis of whole blood samples (most sensitive if blood is collected during the first week of illness; rapidly decreasing in sensitivity when antibodies reach a high level) and PCR or immunohistochemistry of biopsy specimens. Negative PCR results should not rule out the diagnosis, and treatment should not be withheld. Culture isolation of *C. burnetii* is possible in specialized laboratories only, as routine hospital blood cultures cannot detect the microorganism. The gold standard serologic test for diagnosis of Q fever is the indirect immunofluorescence assay (IFA), performed on paired serum samples collected the first as early in the disease as possible (preferably in the first week of symptoms), and the second 2 to 4 weeks later. The first IgG IFA titer should therefore be typically low or negative, and the second should instead exhibit a significant (four-fold) increase. Analysis of two samples is necessary as antibodies to *C. burnetii* may remain elevated for months or longer after the disease has resolved, or may be detected in persons previously exposed to antigenically related organisms, therefore, interpretation of one sample may be difficult. Humans can develop antibody response against both *C. burnetii* phase I and phase II. In particular, in acute infection, an antibody response to *C. burnetii* phase II antigen is predominant and is higher than phase I antibody response; the reverse is true in chronic infection which is associated with a rising phase I IgG titer (according to current U.S. case definitions >1:800) that is often much higher than phase II IgG (NASPHV & CDC, 2011).

For animal diagnosis in the context of serial abortions and stillbirths, samples should be collected from aborted fetuses, placenta and vaginal discharges soon after abortion or parturition. The diagnosis should always include a differential investigation of major abortive agents. Early detection of a Q fever outbreak of abortions in a herd or flock and correct biocontainment measures are essential to prevent and limit both environmental and farm-based routes of infection. A positive case is a herd or flock with clinical signs (abortion and/or stillbirth) for which the presence of the agent has been confirmed. As a rule, in the veterinary practice, a breeding or a flock can be considered clinically affected by Q fever if three circumstances occur: abortions or stillbirths, presence of *C. burnetii* in samples from affected animals (evaluated by Quantitative PCR analysis) and presence of seropositive animals (evaluated by ELISA test). EFSA criteria (Sidi-Boumedine et al., 2010) suggested for a correct diagnosis of *C. burnetii* as abortive agent in bovine herds and ovi-caprine flocks are summarized in the figures 3 and 4, respectively.



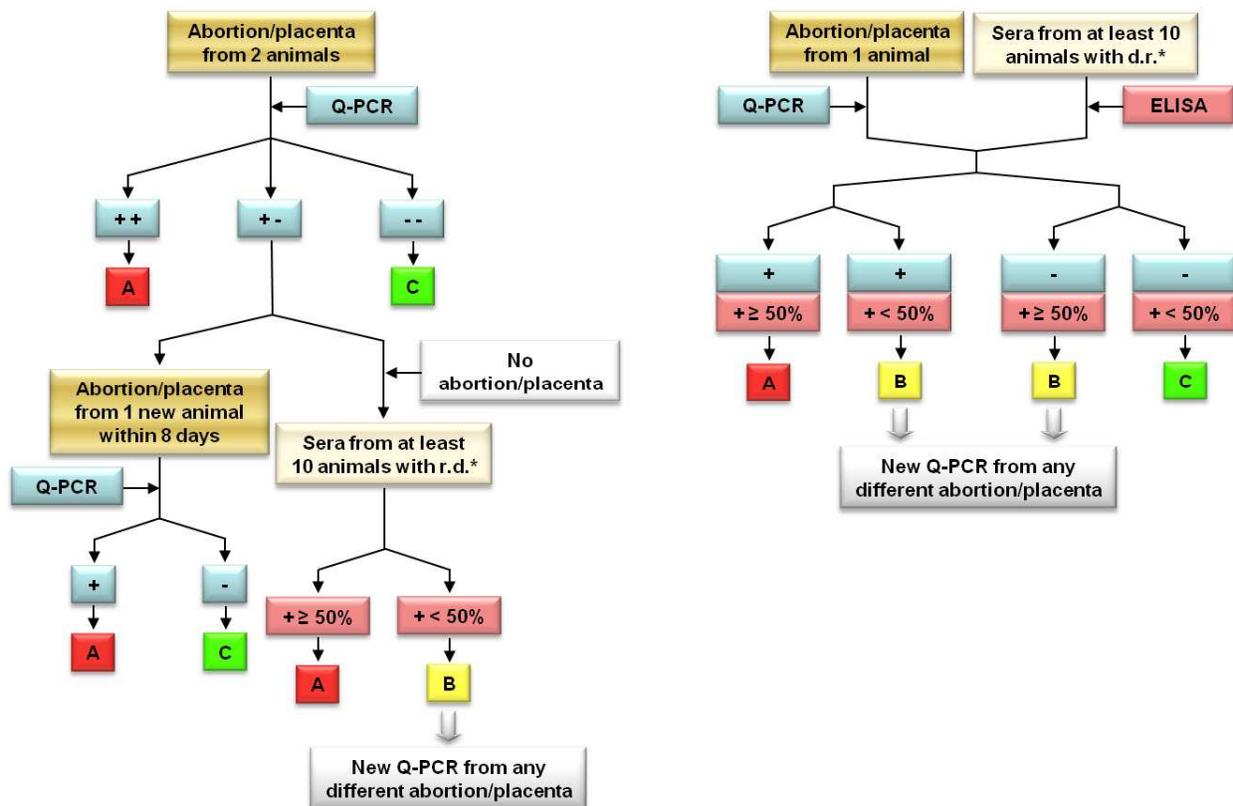
- A** The herd can be considered as clinically affected
- B** Q fever cannot be excluded at the herd level
- C** *C. burnetii* is not the cause of the abortion at the herd level
- N.D.** Unclear diagnosis
- Q-PCR** Quantitative Real-Time PCR: proposed threshold: 10⁴ cfu/gr; for pooled samples: 10³ cfu/gr
- r.d.* Animals with reproductive disorders

Fig. 3. EFSA criteria for a correct diagnosis of Q fever in bovine herds

7.1 Direct techniques

For specific laboratory investigations, it may be necessary to isolate the agent. The direct methods for the isolation and the identification of the infectious agent require proper samples, mainly represented by placenta, vaginal mucus, milk, colostrum, feces and tissues of the aborted foetus, such as liver, lung and content of the stomach, collected immediately after the abortion.

Several techniques are available for *C. burnetii* identification (OIE, 2010), even if they are often characterized by low specificity.



- A** The herd/flock can be considered as clinically affected
B Q fever cannot be excluded at the herd/flock level
C *C. burnetii* is not the cause of the abortion at the herd/flock level
Q-PCR Quantitative Real-Time PCR: proposed threshold: 10^4 cfu/gr; for pooled samples: 10^3 cfu/gr
 r.d.* Animals with reproductive disorders

Fig. 4. EFSA criteria for a correct diagnosis of Q fever in ovi-caprine flocks

7.1.1 Staining techniques

These techniques can be carried out in case of abortions suspected to have an infectious origin. Best results can be obtained by smears of placental cotyledon prepared on microscope slides, but lung, liver and abomasum contents of the aborted foetus or vaginal discharges may be used as well. Several methods are suitable for *Coxiella* identification: Stamp, Gimenez, Macchiavello, Giemsa and modified Koster (Gimenez, 1964; Quinn et al., 1994; Samuel & Hendrix, 2009). The first three techniques give the best results (OIE, 2010). These methods are close to the modified Ziehl-Neelsen method involving basic fuchsin to stain bacteria. The Stamp method is preferred in veterinary laboratories whereas the Gimenez method is very fast for monitoring infected cultural cells in research laboratories. Attention must be taken in the interpretation of the results as, microscopically, *C. burnetii* can be confused with *Chlamydophila abortus* or *Brucella* spp. When biological staining is inconclusive, one of the other methods may be used as a confirmatory test.

7.1.2 Isolation of the agent

These techniques have been abandoned because of their high risk level, even if they can be necessary when isolation of *Coxiella* is required from samples contaminated with more than a bacterial species (OIE, 2010). When *C. burnetii* is present in large numbers and is combined with a low contamination rate with other bacteria, direct isolation by inoculation of embryonated chicken eggs or cell culture is possible (Maurin & Raoult, 1999; Samuel & Hendrix, 2009). A cell microculture system from a commercially available method used for virus culture, the shell vial cell culture, has been adapted for isolating strict or facultative intracellular bacteria, including *C. burnetii*. Such a method was described for *C. burnetii* in 1990 (Raoult et al., 1990). Suspensions of samples are inoculated into a cell line to observe the characteristic vacuoles of *C. burnetii* multiplication. This method was developed for humans but could be adapted for animals. With heavily multi-contaminated samples, such as placentas, vaginal discharges, feces, or milk, the inoculation of laboratory animals may be necessary as a filtration system. Mice and guinea-pigs are the most appropriate animals for this purpose (Scott et al., 1987).

7.2 Indirect techniques

Indirect techniques include the indirect immunofluorescence assay (IFA), the ELISA test and the complement fixation test (CFT) to be performed on serum samples, or, in the case of the ELISA, also on milk samples. Such methods result useful for the screening of high numbers of samples, as in the case of entire herds/flocks, but do not provide clear results for single animal investigations. In fact some animals can remain seropositive for quite several years following acute infection, other animals can shed *C. burnetii* before seroconversion and therefore represent a risk factor for infection, while other animals never seroconvert (Maurin & Raoult, 1999). To the moment tests able to discriminate between infected and vaccinated animals do not exist yet.

7.2.1 Indirect Immunofluorescence Assay (IFA)

In human medicine, the IFA adapted as a micro-immunofluorescence technique is the current method for the serodiagnosis of Q fever (Tissot-Dupont et al., 1994). Both phase I and phase II *C. burnetii* antigens are used. The two forms of the infection, acute and chronic, have different serological profiles: during acute Q fever, IgG antibodies are elevated against phase II only whereas during chronic Q fever, high levels of IgG antibodies to both phase I and II of the bacteria are observed (Tissot-Dupont et al., 1994).

7.2.2 Complement Fixation Test (CFT)

The test detects complement-fixing antibodies present in the serum. The CFT is specific but less sensitive than the ELISA or IFA (Kittelberger et al., 2009; Rousset et al., 2007; 2009). The CFT is still used by laboratories in many countries. This method often uses antigen in phase II prepared from a mixture of two strains (Nine Mile and Henzerling) or a mixture of antigens in phase I and II prepared from Nine Mile strain.

7.2.3 Enzyme-Linked Immunosorbent Assay (ELISA)

This technique has a high sensitivity and a good specificity (Kittelberger et al., 2009; Rousset et al., 2007; 2009). It is easy to perform. The ELISA is preferred to IFA and CFT, particularly

for veterinary diagnosis, because it is convenient for large-scale screening and, is a reliable technique for demonstrating *C. burnetii* antibody in various animal species (Jaspers et al., 1994; Soliman et al., 1992). Ready-to-use kits are commercially available and can detect anti-phase II antibodies or both anti-phase I and II antibodies.

7.3 Specific detection methods

Detection of *C. burnetii* in samples can also be achieved by specific immunodetection (capture ELISA, immunohistochemistry), in-situ hybridisation or DNA amplification (Jensen et al., 2007; Samuel & Hendrix, 2009; Thiele et al., 1992). Immunohistology may be used with paraffin-embedded tissues or on acetone-fixed smears (Raoult et al., 1994). The method is an indirect immunofluorescence or immunoperoxidase assay using polyclonal *C. burnetii* specific antibodies. Fluorescent in-situ hybridisation using specific oligonucleotide probes targeting 16s rRNA may be used on paraffin-embedded tissues, especially placenta samples (Jensen et al., 2007).

C. burnetii detection is today mainly performed by PCR. This method has many advantages because it is highly specific and sensitive, it allows the inactivation of the microorganism by heating to 90°C for 30-60 min, it allows working on different kinds of samples without the need to isolate the bacterium. Unfortunately this method does not allow the isolation of the agent. Target sequences for PCR are numerous, and the most used is the IS1111 (accession number M80806), which renders the technique even more sensitive as this insertion sequence is broadly repeated in the genome of *Coxiella*. PCR is therefore an effective method for the identification of shedding animals. Recently a Real-Time PCR protocol has been set up for the identification and the quantification of the number of bacteria present in a biological matrix. The quantification of *C. burnetii* in the abortion products is an extremely important information as it is the core part of a correct diagnosis of this pathogen as the real cause of the abortion (Sidi-Boumedine et al., 2010).

7.4 Genotyping methods

Detection, isolation and identification of *C. burnetii* can be completed with a molecular characterization, useful for epidemiological studies. 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) (Jäger et al., 1998), and sequence and/or PCR-RFLP (Restriction Fragment Length Polymorphism) analysis of *icd*, *com1* and *mucZ* genes. More recently, the two PCR-based typing methods 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) are gaining importance for several reasons. Indeed they permit the typing of *C. burnetii* without the need for isolation of the organism. Moreover they exhibit high discriminating power with relatively low costs. MLVA analysis is currently the reference method for the genetic characterization of important pathogens such as *M. tuberculosis*, *B. anthracis* and *Y. pestis*. Recent studies have shown that the application of this technique to *C. burnetii* isolates both of animal and human origin allowed the identification of 36 different genetic profiles on a total of 42 isolates (Arricau-Bouvery et al., 2006). Moreover, databases have been established, <http://minisatellites.u-psud.fr/MLVANet/> and <http://ifr48.timone.univ-mrs.fr> for MLVA and MST, respectively. The availability of such databases allows easy

inter-laboratory comparisons which might lead to a better understanding of the propagation of *C. burnetii* isolates.

These tools are very useful for epidemiological investigation, particularly to clarify links regarding sources of infection, for better understanding of the epidemiological emerging factors, and to a lesser extent, for evaluating control measures.

8. Therapy and prophylaxis

In man doxycycline is the first line treatment for all adults, and for children with severe illness. Doxycycline is most effective at preventing severe complications if it is started early in the course of disease. Failure to respond to this antibiotic indicates that *C. burnetii* is not the aetiological agent of the illness, as resistance to doxycycline has never been documented. The recommended dosage for acute Q fever in adults is 100 mg every 12 hours, while for children under 45 kg is 2.2 mg/kg of body weight given twice a day. Standard duration of treatment is 2-3 weeks, or for at least three days after the fever subsides, or until there is evidence of clinical improvement. The recommended treatment for adults affected by chronic Q fever includes 100 mg of doxycycline every 12 hours and 200 mg of hydroxychloroquine every 8 hours. Standard duration of treatment is 18 months (NASPHV & CDC, 2011).

In animals little information is available on the effectiveness of antibiotic treatments, which are often used for reducing the number of abortions and the level of elimination of *C. burnetii* during parturition. Antimicrobial treatment is in fact used mainly to minimize shedding of the organism in the placenta and birth fluids rather to eliminate it. This treatment doesn't prevent entirely neither the abortion (Berri et al., 2005a), nor the elimination of *Coxiella* during parturition (Arricau-Bouvery & Rodolakis, 2005). The prophylaxis based on antibiotic treatment provides therefore the advantage to reduce the risk of abortion, but it doesn't determine the eradication of the disease. In fact, following antibiotic treatment, the animals can still shed *Coxiella* even if they result clinically recovered from the disease.

During Q fever outbreaks the spreading of *Coxiella* in the herd can be prevented or at least reduced by applying control measures including severe hygiene protocols, aiming to prevent environmental contamination (manure composting, fight against natural reservoirs, separation of the areas used for parturition and new born, ready elimination of vaginal discharges and abortion products) and possible culling of seropositive and/or shedding animals.

Vaccination seems to be the only efficient strategy for the control of the disease. Currently two typologies of vaccines exist, one developed against the phase I (virulent), and one against the phase II (avirulent). The commercial available products are Q-VAX® (for human use), Coxevac® (for veterinary use) and Chlamivax FQ® (for veterinary use, developed also against *Chlamydophila abortus*). The use of vaccine does not avoid the risk of shedding of the microorganism from infected animals. Vaccination is therefore suitable only for seronegative herds/flocks. The use of the anti-phase I vaccine in the veterinary practice results useful not only for the control of the disease inside the herd/flock, but also effective against the spreading of the infection in the neighbouring herds as well as in man.

9. Conclusion

The numerous cases of Q fever in animals and in man recently occurred in the Netherlands renewed the interest of the sanitary authorities and of the scientific community on a disease for too long neglected. The presence of this pathology worldwide and the underestimation of this disease highlight the need of specific serological and molecular investigations including careful examination of all the infertility and abortion cases recorded in domestic ruminant herds/flocks. Epidemiological data should be used to set up effective monitoring and prophylaxis strategies for the control of Q fever. Particular attention should be posed on the control of this pathology in domestic and wildlife animals, aiming to contain the economic and sanitary impact that the uncontrolled spread of Q fever would have on human health.

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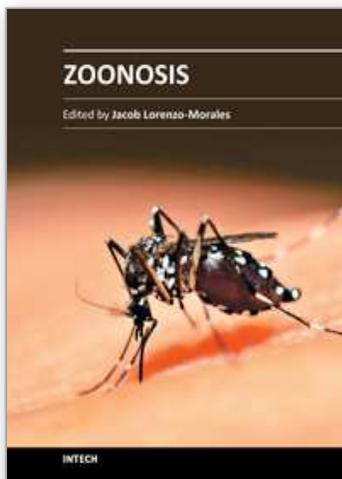
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Zoonosis

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Zoonotic diseases are mainly caused by bacterial, viral or parasitic agents although "unconventional agents" such as prions could also be involved in causing zoonotic diseases. Many of the zoonotic diseases are a public health concern but also affect the production of food of animal origin thus they could cause problems in international trade of animal-origin goods. A major factor contributing to the emergence of new zoonotic pathogens in human populations is increased contact between humans and animals. This book provides an insight on zoonosis and both authors and the editor hope that the work compiled in it would help to raise awareness and interest in this field. It should also help researchers, clinicians and other readers in their research and clinical usage.

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