Q FEVER – STILL A QUERY AND UNDERESTIMATED INFECTIOUS DISEASE

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Summary. – *Coxiella burnetii* (*C.b.*) is a strictly intracellular, Gram-negative bacterium. It causes Q fever in humans and animals worldwide. The animal Q fever is sometimes designated "coxiellosis". This infection has many different reservoirs including arthropods, birds and mammals. Domestic animals and pets, are the most frequent source of human infections. Q fever may appear basically in two forms, acute and chronic (persistent). The latter form of Q fever in animals is characteristic by shedding *C.b.* into the environment during parturition or abortion. Human Q fever results usually from inhalation of contaminated aerosols originating mostly from tissue and body fluids of infected animals. Q fever may appear in humans either in an acute form accompanied mainly by fever (pneumonia, flu-like disease, hepatitis) or in a chronic form (mainly endocarditis). Diagnosis of Q fever is based on isolation of the agent in cell culture, its direct detection, namely by PCR, and serology. Detection of high phase II antibodies titers 1–3 weeks after the onset of symptoms and identification of IgM antibodies are indicative to acute infection. High phase I IgG antibody titers >800 as revealed by microimmunofluorescence offer evidence of chronic *C.b.* infection. For acute Q fever, a two-weeks-treatment with doxycycline is recommended as the first-line therapy. In the case of Q fever endocarditis a long-term combined antibiotic therapy is necessary to prevent relapses. Application of Q fever vaccines containing or prepared from phase I *C.b.* corpuscles should be considered at least for professionally exposed groups of the population. Infections caused by *C.b.* are spread worldwide and may pose serious and often underestimated health problems in human but also in veterinary medicine. Though during the last decades substantial progress in investigation of *C.b.* has been achieved and many data concerning this pathogen has been accumulated, some questions, namely those related to the pathogenesis of the disease, remain open.

Key words: Q fever; epidemiology; clinical presentation; pathogenesis; immunity; diagnosis; treatment; prevention

History

Derrick was the first to recognize Q fever as a new entity in 1937 as a result of his study of an outbreak of a febrile disease among abattoir workers in Brisbane, Australia (Derrick, 1937). Thorough clinical studies resulted in the description of the disease but serological examination for antibodies to numerous pathogens gave negative results. It was understood that the investigators were dealing with a new type of the disease, which had not been previously recognized. Derrick designated the disease Q (query) fever to illustrate its uncertain etiology (Derrick, 1939). Later on, this agent was identified as rickettsia and was named by Philip (1948) *C.b.*; a name dedicated to Cox and Burnet, who contributed essentially to the identification of the Q fever agent (Davis and Cox, 1938).

The first recognized epidemics established Q fever as a disease of previously unsuspected importance. Q fever was

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Abbreviations: *C.b.* = *Coxiella burnetii*; CF = complement fixation; EIA = enzymatic immunoassay; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; IFN = interferon; IFT = immunofluorescent technique; LPS = lipopolysaccharide; MA = microagglutination; MAb = monoclonal antibody; PCR = polymerase chain reaction; TNF = tumor necrosis factor

found during the Second World War when outbreaks of the disease have occurred in the Mediterranean area in the Balkans ("Balkan Grippe") among German (Imhauser, 1949) and allied troops (Robbins and Ragan, 1946). The Balkans thus became the territory in which *C.b.* could circulate and be spread to other parts of Europe; e.g., Q fever was probably introduced to Slovakia by infected sheep from Roumania (Řeháček and Tarasevich, 1988). Isolation of *C.b.* from the cow's placenta and further epidemiological and epizootological studies clearly indicated a zoonotic nature of this disease (Babudieri, 1953). In the 50-ties, Q fever was described to occur already in fifty countries of five continents (Kaplan and Bertagna, 1955). During the next 3–4 decades *C.b.* spread almost over the whole Europe (Řeháček and Tarasevich, 1988). At present, it occurs throughout the world (Kazár, 1999), except for New Zealand (Hilbink *et al.*, 1993).

Bacteriology

C.b. is a small Gram-negative obligate intracellular microorganism (from 0.2 to 0.4 μm wide and from 0.4 to 1 μm long). Based on nucleotide sequencing of the gene encoding 16S rRNA it was shown that this bacterium belongs to a separate genus *Coxiella* of the gamma subdivision of *Proteobacteria* (Weisburg *et al.*, 1989; Stein *et al.*, 1993). *Coxiella* has been placed into the order *Rickettsiales*, family *Rickettsiaceae* and tribe *Rickettsiae* together with genera *Rickettsia* and *Orientia* (Maurin and Raoult, 1999). Phylogenetically it is most closely related to the genera *Legionella* and *Francisella* (Maurin and Raoult, 1999).

In several *C.b.* islates no differences in the 16 S rRNA have been observed. Based on DNA-DNA hybridization, *C.b.* expressed a low heterogeneity among strains (Vodkin *et al.*, 1986). Earlier, by examination of 38 isolates of *C.b.* by restriction fragment length polymorphism analysis, six genomic groups were distinguished (Hendrix *et al.*, 1991). Further analysis of *C.b.* DNA restriction fragments by pulsed-field gel electrophoresis with *Not*I and *Sfi*I resulted in distinguishing four genotypes of strains, which originated from the USA (Heinzen *et al.*, 1990). Sixteen further genotypes have been described by use of this method for identification of 80 isolates collected in various parts of the world (Sekeyová *et al.*, 1996; Jäger *et al.*, 1998). Sekeyová *et al.* (1999) have investigated intraspecies genetic diversity by sequence comparison of a 715 bp region of the Com1 gene and a 774 bp region of the *Muc*Z gene in 37 strains of *C.b.* isolated from animals and humans in various parts of the world and have found five and four groups, respectively. This grouping reflected neither the relation to the geographical distribution nor the relation to the disease form. Whereas the groups I, III, IV and V contained both human and animal isolates, the group II consisted of human isolates only.

Four different plasmids varying in size were described for *C.b.* (Minnick *et al.*, 1990; Savinelli and Mallavia, 1990; Valková and Kazár, 1995). Comparison of nucleotide sequences of the QpH1 plasmids from *C.b.* in phase I and II, and that of the plasmid integrated into the chromosome of the plasmidless *C.b.* strain, revealed no evidence for specific genes or sequences involved in phase variation (Thiele *et al.*, 1994; Willems *et al.*, 1997). Though the plasmid type and size were related to the form of the disease, acute versus chronic (Mallavia, 1991), further studies did not confirm this correlation (Yu and Raoult, 1994; Thiele and Willems, 1994). Predisposing host factors probably play a major role in the clinical form of the disease, but the route of infection should also be considered (Marrie *et al.*, 1996; LaScola *et al.*, 1997).

Attention should be also paid to a phase variation of *C.b.* It has, a cell wall resembling that of Gram-negative bacteria. When propagated in non-immunoincompetent cell cultures or hen eggs, *C.b.* converts from a naturally occurring phase I to a laboratory acquired phase II. This phase variation is analogous to the smooth-rough (S-R) variation of the family *Enterobacteriaceae* (Baca and Paretsky, 1983). Such process is accompanied by a change of physicochemical and biological properties of the agent (Kazár *et al.*, 1974; Moss and Hackstadt, 1987) and by dramatic changes in LPS structure (Quevedo Diaz and Lukáčová, 1998; Ftáček *et al.*, 2000; Toman, 1999). Chemical composition of LPS changes in correlation with phase variation (Toman, 1992; Škultety and Toman, 1996). The LPS of *C.b.* has an endotoxic activity 100 to 1000 times lower than that of LPS of *Enterobacte-riaceae* (Amano *et al.*, 1987). Antigenic changes in *C.b.* strain Nine Mile were analyzed with monoclonal antibodies (MAbs) against LPS and monitored during passaging in cell cultures. (Hotta *et al.*, 2002). Using such an approach, these authors have found that four stages could be differentiated in *C.b.*

C.b. does not grow in axenic media. It can therefore be isolated only in living substrates, e.g. laboratory animals, embryonated eggs and cell cultures. In the developmental cycle of *C.b.*, both vegetative growth and spore-like formation occur (McCaul *et al.*, 1994). It lives and multiplies by binary fission in the phagolysosomes of susceptible cells at acidic pH (Baca *et al.*, 1994). It is known to occur in two morphologically distinct forms: – a more metabolically active large cell variant and a less metabolically activity small cell variant dividing by binary fission (McCaul *et al.*, 1991; Heinzen *et al.*, 1999). Several proteins synthesized by these forms have been characterized (Heinzen *et al.*, 1999). Spore-like forms have been described in large cell variant forms (McCaul *et al.*, 1991) and detected in *C.b.*-infected cardiac valves. But they have not been isolated in a pure form (Norlander, 2000). Recent results of LaScola and Raoult

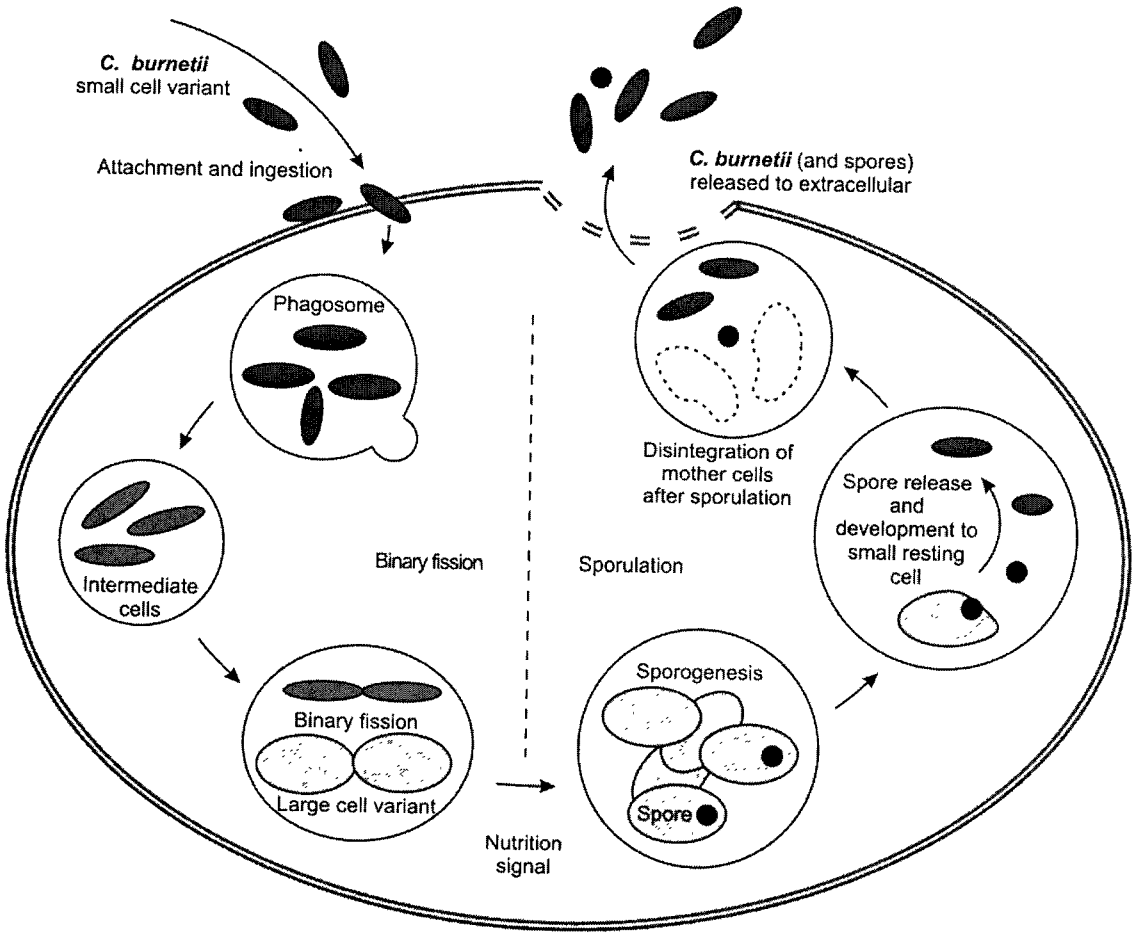

Fig. 1
Developmental cycle of *C.b.* occurring within the phagolysosomes of eukaryotic cell
Small and large cell variants and spores are formed during the cycle (Williams, 1991).

(2001) have indicated that free-living amoebae (e.g. *Acathamoeba castellanii*) can provide an intracellular niche for the spore formation and survival of *C.b.* in the environment. Developmental cycle of *C.b.* is outlined in Fig. 1.

Geographical distribution

Q fever is an infectious disease described worldwide (Maurin and Raoult, 1999) that occurs in all geographic and climatic zones, where the conditions of natural *C.b.* infection and circulation exist (Marrie, 1990; Khavkin, 1991; Kováčová *et al.*, 1996; Hirai and To, 1998; Zhang *et al.*, 1999). New Zealand is probably the only larger country without Q fever (Hilbink *et al.*, 1993).

In Europe, Q fever is most frequent in France (Maurin and Raoult, 1999), Spain (Tellez *et al.*, 1988; Velasco, 1996), Portugal (Bacellar *et al.*, 1991), United Kingdom (Pebody *et al.*, 1996), Germany (Hellenbrand *et al.*, 2001), Italy (Manfredi-Selvaggi *et al.*, 1996), Greece (Tselentis *et al.*, 1995), the Netherlands (Houvers and Richardus, 1987), Sweden (Akesson *et al.*, 1991; Macellaro *et al.*, 1993), Poland (Anusz, 1995), the former Czechoslovakia (Literák and Řeháček, 1996), and Bulgaria (Serbezov *et al.*, 1999). Occurrence of this disease is obvious also in other European countries, where small outbreaks have occured (Rady *et al.*, 1987; McQuiston *et al.*, 2002).

In Slovakia, Q fever is known since 1954 when outbreaks occurred among agricultural workers who contracted the infection from sheep and among workers of a textile plant who were exposed to *C.b.*-contaminated imported cotton (Řeháček and Tarasevich, 1988). From that time until the 80ties, waves of epizootics and small epidemics have appeared in factories processing cotton, wool and hides.

Since that time only sporadic cases of Q fever have been reported from different parts of the country. In a survey covering 1989–1996, out of 21,197 human sera tested 655 (3%) reacted with the phase II *C.b.* antigen and acute Q fever was diagnosed in 23 cases (0.1%) only. At the same time, *C.b.* antibodies were detected in 11% of cattle and in 3% each of sheep and goats. An exception was an explosive epidemic of Q fever in 1993 that affected 103 humans (Varga, 1997; Kováčová *et al.*, 1998). However, since in many countries the reporting on Q fever cases is not required and many *C.b.* infections are inapparent (Dupuis *et al.*, 1987), it is difficult to estimate the number of cases of the disease that actually did occur (Marrie and Raoult, 1997).

New cases of Q fever have been recognized in countries in which sufficient attention has been paid to its diagnosis, namely in Scandinavia (Akesson *et al.*, 1991) and Japan (Hirai and To, 1998) in the 80ties and 90ties and recently in Turkey (Cetinkaya *et al.*, 2000) and in Oman (Scrimgeour *et al.*, 2002).

Epidemiology

The basic cycle of *C.b.* circulation involves many species of wildlife animals and their ectoparasites; the second cycle proceeds in domestic animals (Aitken, 1989). Wildlife Q fever is qualified as an arthropod-borne infection (Lang, 1990). Ticks are principal vectors and reservoirs of *C.b.* The agent was found to infect more than forty species of ticks belonging to 12 genera, but their role in direct transmission of the pathogen to humans is not important (Parola and Raoult, 2001). Feces of ticks contain numerous viable *C.b.* corpuscles where they may persist for a long time. The tick species that are the most frequent vectors of *C.b.* belong to the genera *Ixodes, Rhipicephalus, Amblyomma* and *Dermacentor* (Parola and Raoult, 2001).

Ticks transmit the agent vertically, i.e. transstadially and transovarially to their progeny and horizontally via bite or feces to wild animals, especially rodents and birds, thus causing wildlife Q fever (coxiellosis). The agent multiplies in the cells of the middle gut and stomach of ticks. Transmission by ticks to domestic animals results in livestock Q fever that can be maintained later on by a direct contact of animals or via their excrements. The wildlife Q fever is a tick-borne infection; other hematophagous arthropods, e.g. fleas, bugs, lice and mites serve probably as mechanical vectors. On the other hand, human Q fever is an air-borne infection, usually via inhalation of contaminated aerosols. Only two cases of Q fever are suspected to be contracted by a tick bite and co-infection with *Rickettsia conorii* and *C.b.* was also described (Eklund *et al.*, 1947; Beaman and Hung, 1989; Janbon *et al.*, 1989).

Every tick species parasitizing on a susceptible host can be suspected to harbor and spread *C.b.* in a known area of endemicity. Very important reservoirs of *C.b.* in nature are small wild rodents, but the infection has been demonstrated also in insectivores, lagomorphs, carnivores, bats and even reptiles and fish (Sawyer *et al.*, 1987; Řeháček and Tarasevich, 1988).

Animal Q fever (coxiellosis)

In livestock Q fever, the primary reservoirs of *C.b.* are cattle, sheep and goats; they represent also the most frequent source of human infection (Marrie, 1990). Reactivation of infection in females during pregnancy is accompanied by shedding of great amounts of the infectious agent into the environment. Transmission of *C.b.* among cattle, sheep and goats is mostly associated with parturition or abortion. Animals become infected with *C.b.* by contaminated aerosol and/or by the bite of ticks that carry the agent (Parola and Raoult, 2001).

Cattle. The infection starts with rickettsemia causing acute infection, which later develops into a chronic form. The illness rarely develops from a latent infection and can be maintained as a chronic infection in dairy cows and goats but not in sheep (Lang, 1990). No correlation has been reported between the *C.b.* infection and abortion in these animals. A prolonged presence of specific antibodies in the sera of cows and shedding of the agent in the milk compared with sheep has been reported (Brooks *et al.*, 1986; Lang, 1990). Calves, which have ingested infectious milk eliminate the agent in the feces and thus contribute to the spread of infection. The strains of *C.b.* isolated from cow milk in the former Czechoslovakia (Kocianová *et al.*, 2001) were less virulent for laboratory animals than those isolated from ticks.

Sheep. They are generally known to be excellent disseminators of *C.b.* during delivery or abortion. These animals have been implicated as the source of infection in 24 of 40 documented outbreaks in Germany since 1947 (Hellenbrand *et al.*, 2001). Naturally infected sheep showed usually a latent infection, which may develop into a manifest disease during the pregnancy (Berri *et al.*, 2002). Frequent Q fever infections have been observed along the route of sheep flocks (Hrabar *et al.*, 1971). A great Q fever epidemic involving 415 serologically confirmed human cases has been recorded in Switzerland (Dupuis *et al.*, 1985). A great Q fever outbreak has occurred also in West Midlands with 417 diagnosed human cases (Smith *et al.*, 1993; Hawker *et al.*, 1998). The most likely source of infection was a farmland with a large number of parturient sheep from which the surrounding area was contaminated by *C.b.* via wind (Hawker *et al.*, 1998).

Goats. They develop an acute as well as a chronic latent infection demonstrated by fever, bronchopneumonia, mastitis, cessation of lactation and abortions. However,

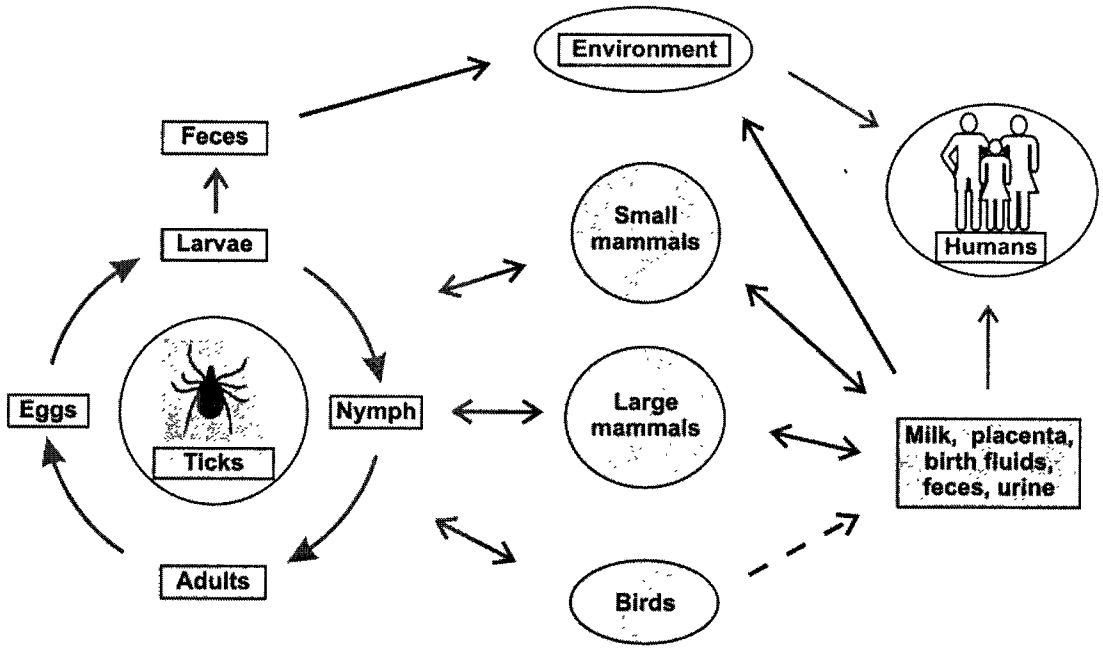

Fig. 2
Circulation of *C.b.* in nature
The main cycle.

abortion in goats and sheep but not in cows has been linked to *C.b.* infection (Lang, 1990). Though the agent is excreted in milk, urine and feces, the most important is the fact that it is shed in high numbers and for a long period via the amniotic fluid and placenta of infected animals (Little, 1983; Moore *et al.*, 1991).

Recently due to an increased number of human cases of Q fever in territories from which they have not been previously reported (Hatchette *et al.*, 2001), more attention has been paid to the role of goats in the circulation of *C.b.* Goats have been implicated in outbreaks of Q fever in many countries and have replaced sheep and cattle as the most common source of human infections in Bulgaria (Serbezov *et al.*, 1999). The infection has been noted in other animals including domesticated pets, mostly cats and dogs, which live in close contact with humans (Langley *et al.*, 1988; Morita *et al.*, 1994; Matthewman *et al.*, 1997; Buhariwalla *et al.*, 1996).

Dogs. By their way of life, namely frequent hosts of ticks, settlement in an environment contaminated with *C.b.*, they may be considered to have a role in the *C.b.* ecology. A relatively high seroprevalence has been reported (Werth *et al.*, 1987; Kocianová *et al.*, 1992); the highest level of antibodies was always found in stray animals.

Cats. They are infected with the Q fever agent during the time of parturition and become the source of infection for humans as described for the maritime provinces of Canada (Marrie *et al.*, 1988).

Birds. They may also be infected with *C.b.* via exposure to feces and ticks containing *C.b.* The agent has been isolated from pigeons, chickens, ducks and geese (Kocianová *et al.*, 1993; To *et al.*, 1998; Stein and Raoult, 1999). Humans may acquire Q fever from domestic poultry (Řeháček and Tarasevich, 1988). The fact that birds are able to spread *C.b.* over long distances via their feces and ectoparasites opens new ways for explanation of the epidemiology of Q fever (Řeháček and Tarasevich, 1988) (Fig. 2).

Human Q fever

It is is an air-borne infection contracted by inhalation of aerosolized *C.b.* corpuscles from contaminated environment. Humans are very susceptible to the infection and very few *C.b.* corpuscles present in dried placental and birth materials or excretments from infected animals are enough to cause infection (Ormsbee *et al.*, 1978).

The alimentary route, namely ingestion of contaminated milk or milk products is a less common mode of transmission (Marrie, 1990). In earlier studies it has been assumed that ingestion of milk leads to seroconversion only. It has been noted, however, that consumption of unpasterized goat dairy

products causes Q fever in humans (Fishbein and Raoult, 1992). The predominance of Q fever hepatitis in rural France was linked to a more frequent drinking of raw milk (Tissot-Dupont *et al.*, 1992). Importance of the digestive route in the human infection was minimized by the necessity to administer 10,000 times more *C.b.* particles by oral than by intraperitoneal route to cause infection in mice (Durand, 1993).

Other modes of transmission to humans, e.g. person-to-person transmission during autopsy, delivery or abortion, are very rare and epidemiologically unimportant (Mann *et al.*, 1986; Marrie, 1990; Raoult and Stein, 1994), including the suggested possibility of sexual transmission (Kruszewska *et al.*, 1996; Milazzo *et al.*, 2001).

Q fever is considered to be primarily an occupational disease of workers in close contact with farm animals or processing products; that explains why it is more frequent in men than women. It may occur in workers in or visitors to areas of risk as research laboratories, abattoirs and cotton processing plants as well as in incidental bystanders (Raoult and Stein, 1994). Abe *et al.* (2001) have found IgG antibodies to *C.b.* using the phase II antigen in 15.5% of veterinarians, but only in 3.6% and 5.1% of blood donors and medical workers, respectively. Students of veterinary medicine represent also a group of risk (Valencia *et al.*, 2000). In many areas the most explosive epidemics have occurred in stockyards, meat packing plants and medical laboratories using sheep for research (Řeháček and Tarasevich, 1988).

C.b. is resistant to heat, drying, osmotic shock, ultraviolet light and common disinfectants (Scott and Williams, 1990); it may survive from months to years in the environment (Kazár and Brezina, 1991). This explains the fact that it can be spread from farmlands to urban areas and to unexpected places; under some conditions it can be considered a wind-borne infection (Hawker *et al.*, 1998; Tissot-Dupont *et al.*, 1999). Consequently, a *C.b.* aerosol can be used in biological warfare and is considered a potential terrorist threat (Rotz *et al.*, 2002).

Clinical presentation

Q fever is a highly variable disease ranging from acute to fatal chronic infection, but subclinical and asymptotic infections are also common (Dupuis *et al.*, 1987; Choi, 2002). Incubation period for Q fever varies from 1 to 3 weeks depending on the number of *C.b.* particles initially infecting humans (Maurin and Raoult, 1999). Acute Q fever resembles nearly any infectious disease. It is manifested mostly as a flu-like disease or atypical pneumonia, less often as hepatitis, isolated and/or prolonged fever, and a bone or exanthematous disease. A neurological involvement is also possible (Bernit *et al.*, 2002). Pericarditis, myocarditis and ocular manifestations are rare, but myocarditis can be a life-threatening condition (Levy *et al.*, 1999; Fournier *et al.*, 2001). Though the acute Q fever is usually a disease with about 1% lethality, complications such as pyurria, spleen rupture, rapid fatal pneumonia, encephalitis, acute renal or congestive heart failure and acute respiratory insufficiency may occur (Kazár, 1999). Development of protracted debility and fatigue syndrome is possible (Marmion *et al.*, 1996). The acute Q fever can occur with a rash and petechiae without signs of pneumonia or hepatitis (van Hensbroek *et al.*, 2000). Though it is not common in the acute Q fever, it was present in 20% of 323 French cases (Tissot-Dupont *et al.*, 1992). In a two-year-old child, Q fever was present with acute pericarditis and chronic hepatitis (Baquerro-Artigao *et al.*, 2002). The first case indicating that acute Q fever may occur as acute pancreatitis was reported from Corsica (Stein *et al.*, 1999).

The chronic Q fever, on the other hand, is a serious, often fatal disease, though its mortality rate dropped over the years from 65% to less than 10% due, obviously, to improved regimes of antibiotic therapy (Maurin and Raoult, 1999). Chronic Q fever develops slowly during several years after primary infection in 1% to 10% of cases, mostly in men over 40 with underlying heart disease and/or impaired immunity. A major clinical picture, endocarditis was reported from only a limited number of countries, despite of worldwide distribution of *C.b.* Much less frequent are infections of vascular grafts or aneurysms, chronic hepatitis, and osteoarticular and chronic pulmonary infections.

Q fever deserves special attention during pregnancy (Raoult *et al.*, 2002) as it causes usually placentitis, death *in uteri* (Dindinaud *et al.*, 1991; Hellmeyer *et al.*, 2002), spontaneous abortion or premature birth (Marrie, 1993). Contact with domestic animals at the time of abortion or delivery is of a major risk to humans for contracting the *C.b.* infection (Stein and Raoult, 1998).

Also children are a group which can be affected by *C.b.* Seroepidemiological studies have shown that children are frequently exposed to this agent, but Q fever is rare. Maltezou and Raoult (2002) have identified 46 published pediatric cases only. Such a small number of cases may reflect the fact that Q fever in children is less frequently symptomatic than in adults and that a milder disease develop in children.

Clinical and epidemiologic characteristics of the 1383 patients, whose final diagnostic was Q fever (acute or chronic), were reviewed by Raoult *et al.* (2000). Authors demonstrated that host factors may play a role in the clinical expression of acute Q fever, and they also confirmed that chronic Q fever is mainly determined by host factors.

Because signs and symptoms of Q fever are not specific to this disease, it is difficult to make an accurate diagnosis without appropriate laboratory testing. Clinical signs including positive findings of chest radiography and tomography (Voloudaki *et al.*, 2000), supplemented with liver and bone marrow biopsy (Rexroth *et al.*, 2000) and

patient history (febrility with a recent contact with parturient animals (Varga, 1997)), may indicate the necessity of appropriate microbiological examination, i.e. isolation or detection of the agent and serological examination (Mauch, 1996).

Pathology, pathogenesis and immunity

Little is known about the pathological process associated with the *C.b.* infection in humans, since most patients recover. The three main symptoms, namely the fever, pulmonary signs and elevated liver enzyme levels can coexist (Maurin and Raoult, 1999). The histopathology of Q fever pneumonia revealed the lung hepatization with interstitial edema, lymphocyte and macrophage infiltration, focal intraalveolar necrosis and hemorrhage (Fournier *et al.*, 1998). In liver biopsy a granulomatous hepatitis (doughnut granulomas) can be detected; it may also occur in the bone marrow. In the Q fever endocarditis, vegetation on the surface of the valves is typical (Fournier *et al.*, 1998).

Pathogenesis of Q fever is determined both by the properties of the agent and reactivity of the host (Raoult, 1990). As to *C.b.* pathogenic factors, the LPS, acid phosphatase, superoxide dismutase, catalase, com1 protein, surface proteins and macrophage infectivity potentiator have been suggested (Baca and Mallavia, 1997). Since the physical and genetic maps of *C.b.* are known (Willems *et al.*, 1998), we can expect further pathogenetic factors and gene products, namely heat shock proteins, as reviewed by Norlander (2000). Moreover, *C.b.* can persist indefinitely at unknown sites (Hackstadt, 1990; Harris *et al.*, 1996; Lovey *et al.*, 1999). The route of infection, respiratory vs. oral, can also determine the clinical manifestation of Q fever (Marrie *et al.*, 1996; LaScola *et al.*, 1997). As to the Q fever endocarditis, its pathophysiology is characterized by suppression of the antigen-specific cell-mediated immune response (Mege *et al.*, 1997). It occurs mostly in immunosupressed humans (organ transplant recipients and patients with cancer, lymphoma, chronic renal insufficiency and AIDS) over 40 and in those with underlying heart disease (Maurin and Raoult, 1999). Immune response plays an essential role in recovery from *C.b.* infection (Kazár, 1988; Mege *et al.*, 1997). Acute infections are associated with an inflammatory response and a protective immune response. Chronic infection results from an inefficient response to *C.b.* as demonstrated by failure of the agent to induce IFN-γ and by the detection of high levels of antibodies to *C.b.* (Mege *et al.*, 1997).

Macrophages have an important function in development of protective immunity. Proimflammatory cytokines-interleukins 1 (IL-1) and 12 (IL-12) and tumor necrosis factor alpha (TNF-α), which is secreted by infected macrophages, are capable of modulating the cellular response. TNF-α is involved in the survival of *C.b.* inside the monocytes of patients with Q fever endocarditis (Dellacasagrande *et al.*, 2000), but how does it affect the response of monocytes is unknown. An increased release of TNF-R75 receptor of 75 K by monocytes is specifically associated with Q fever endocarditis (Ghigo *et al.*, 2000). The synthesis and membrane expression of TNF-R75 was not regulated in response to *C.b.* in the course of the disease.

The role of antibodies in Q fever is controversial. As phagocytes are influenced by antibodies the latters may contribute to the elimination of *C.b.* from the immune host. Moreover, the phase II *C.b.* is more exposed to opsonising antibodies than that in phase I, whose LPS masks *C.b.* surface antigens to the immune system (Hackstadt, 1988).

In vivo and *in vitro* studies showed that IFN-γ and IFN-α play important roles in the host defense processes resulting in elimination of *C.b.* (Turco *et al.*, 1984; Dellacasagrande *et al.*, 1999; Capo *et al.*, 1999). Macrophages infected *in vitro* with a virulent *C.b.* showed an early strong induction of TNF-α and IL-1 (Tujulin *et al.*, 1999). These authors did not find the IL-1 production after stimulation with the phase II LPS. They have postulated that factors others than LPS differ in the two phase variants of *C.b.* and that these differences may account for differences in IL-1α production.

Transcription of genes encoding TNF-α and IL-1 and secretion of respective gene products were significantly higher in monocytes from patients with Q fever endocarditis than those in healthy controls (Capo *et al.*, 1996a). Moreover, in patients with recent endocarditis exhibiting high titers of IgGs against phase I *C.b.*, monocytes released significantly higher levels of TNF-α and IL-1 than those in patients with stabilized endocarditis. Hence, the overproduction of inflammatory cytokines might be a marker of the disease activity (Capo *et al.*, 1999). A high level of TNF-α was found in patients with chronic Q fever endocarditis, while that in acute Q fever cases was lower.

TNF-α activates a cascade of secondary inflammatory mediators, eventually leading to endothelial damage and hemodynamic and metabolic derangement (Bone, 1991). It is considered a pivotal cytokine in inflammation and a main endogenous mediator of septic shock. Our preliminary results showed that LPSs from various strains of *C.b.* stimulated murine macrophages to produce different amounts of TNF-α. The highest producers were strains associated with chronic forms of Q fever (Kubeš *et al.*, 2000). Chronic form of this disease is associated with overproduction of IL-10 and deficient *C.b.* killing by monocytes (Ghigo *et al.*, 2001). These authors have hypothesized that replication of *C.b.* inside monocytes requires a macrophage-inactivating cytokine such as IL-10. This cytokine might be considered a marker of disease relapses that may be used in monitoring the efficiency of the treatment (Capo *et al.*, 1996b).

TNF-γ induces killing of *C.b.* in THP-1 cells and also death of these cells by apoptosis and secondary necrosis.

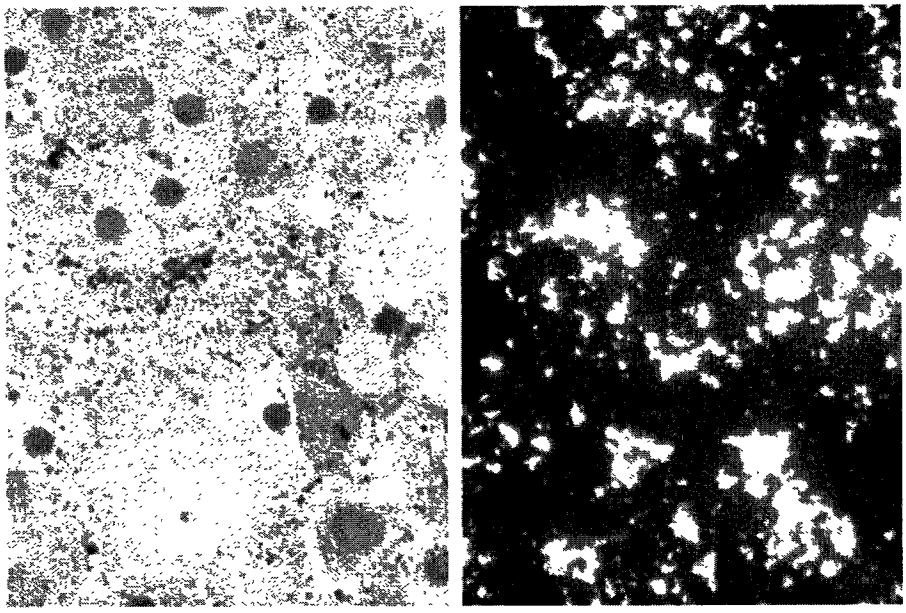

Fig. 3
***C.b.* in phase I propagated in chick embryo yolk sac**
Smears of cells, staining 8 days p.i. by Gimenez (a) Purified phase I *C.b* antigen, IFA test (b). Magnification 720x.

TNF-γ may be useful as an adjuvant treatment of *C.b.*-infected patients (Dellacasagrande *et al.*, 1999).

Laboratory diagnosis

As the signs and symptoms of Q fever are not specific for this disease, it is difficult to diagnose it accurately without appropriate laboratory testing (Houpikian and Raoult, 2002).

Q fever was diagnosed earlier by isolation of the agent in embryonated hen eggs and laboratory animals. However, these procedures were hazardous, required special biosafety cabinets or animal rooms and were restricted to specialized laboratories. Hence, later on, they were replaced by a highly sensitive shell-vial cell culture technique (Marrero and Raoult, 1989; Raoult *et al.*, 1990).

Another possibilities have been a direct detection of *C.b.* in clinical specimens by staining according to Gimenéz or by fluorescence microscopy (Fournier *et al.*, 1998) and different immunoassay techniques (Thiele *et al.*, 1992; Uhaa *et al.*, 1994), especially by use of MAbs (Sekeyová *et al.*, 1996) (Fig. 3).

Electron microscopy is still an important tool for investigation of *C.b.* It can be used in tissue biopsies of patients with persistent Q fever (Isalska *et al.*, 1996; Curry, 2000).

Recently, molecular biology tools, such as polymerase chain reaction (PCR) became important, simple and reliable tools for Q fever diagnosis and detection and differentiation of *C.b.* strains (Mallavia *et al.*, 1990; Willems *et al.*, 1994; Yuasa *et al.*, 1996; Pitcher and Fry, 2000). It was successfully applied to detection of *C.b.* DNA not only in cell cultures but also in clinical specimens; it showed a higher sensitivity than the isolation in cell cultures (Stein and Raoult, 1992; Loranz *et al.*, 1998). Specific, rapid and convenient quantification of *C.b.* DNA was achieved by a colorimetric microtiter plate hybridization assay (Fritz *et al.*, 1995). Recently, universal and species-specific primers have been designed to amplify *C.b.* DNA directly from resected valves (Houpikian and Raoult, 2002).

Serological examination, namely detection of antibodies to *C.b.* still remains the most common way of diagnosing Q fever that should be performed first (Fournier *et al.*, 1998; Kováčová and Kazár, 2000; Houpikian and Raoult, 2002). In this context, existence of two antigenic phases, namely I and II should be mentioned (Stoker *et al.*, 1956). Differentiation of antibodies to either phase antigen is important for precise diagnosis of Q fever and its forms.

In acute Q fever, the levels of antibodies to the phase II antigen are higher than those to the phase I antigen and, in general, the formers are first detected during the second week of the illness (Kováčová *et al.*, 1998). High antibody titers to the phase I antigen (>800) are associated with chronic infection syndrome (endocarditis, bone or valvular and vascular prosthesis infections) without any other clinical or microbiological expansion (Peacock *et al.*, 1983).

In chronic Q fever, a high level of phase I antibodies with a constant or falling level of phase II antibodies together with other signs of inflammatory disease are common. Phase I antibodies appear later in the chronic Q fever and indicate a longer exposure to *C.b.* than in the acute form of the disease. Both types of antibodies can persist for months or years after initial infection (Waag *et al.*, 1995).

The antibody detection depends on the tests and the nature of the antigen used (Kováčová *et al.*, 1998). Since the source and method of preparation of the antigen used may differ among research or diagnostic laboratories, comparison and interpretation of results, namely with sera of domestic animals, is difficult. To overcome this problem, i.e. to obtain comparable results, choice of a proper antigen for a given serological test and animal species and standardization of antigen preparations inclusive of the passage history of *C.b.* are necessary. As an example may serve different cut-off values in the immunofluorescent technique (IFT), which is the most frequent method of choice. It can distinguish between IgA, IgG and IgM antibodies. Detection of IgM, IgA and IgG antibodies improved the specificity of the assay and provided a higher accuracy of diagnosis. IgM antibody levels are helpful in detection of recent infections (Doller *et al.*, 1984; Field *et al.*, 2000).

The indirect ELISA is another test that can be used for serodiagnosis of a *C.b.* infection, both in animals (Behymer *et al.*, 1985) and humans (Kováčová *et al.*, 1987). The assay is highly sensitive, easy to perform, has a great potential adaptability for automatization, can be applied to epidemiological surveys (Field *et al.*, 1983; Rogers and Edlinger, 1986; Embill *et al.*, 1990) and is valuable for diagnosis of both acute and chronic Q fever. Waag *et al.* (1995) have shown that an enzymatic immunoassay (EIA) can be used to diagnose acute Q fever in the case only a single convalescent serum sample is available. The duration of positive response has been found to be longer than five years (Waag *et al.*, 1995). Camacho *et al.* (1998) have found IgA2 antibodies mostly in acute Q fever, while IgG1 antibodies seemed to be typical for the chronic form of the disease.

Of other tests, complement fixation (CF) (Peter *et al.*, 1985) and microagglutination are commonly used. The latter is simple and sensitive and can detect the early antibody response to *C.b.* (Kazár *et al.*, 1981). An indirect hemolysis test can be used for follow-up of the chronic Q fever (Tokarevich *et al.*, 1990).

For evaluation of serological tests a cut off value is very important (Tissot-Dupont *et al.*, 1994). In the CF test, the suggested cut-off value is >32 for the acute Q fever and >200 for the chronic Q fever. In the IFT, a cut off value >200 for phase II IgG antibodies and >50 for phase II IgM antibodies in the acute Q fever but >800 for phase II IgG antibodies in the chronic Q fever are recommended. In ELISA. the proposed cut-off value is >1024 for phase II IgG antibodies and >512 for phase II IgM antibodies.

Cross-reactions of *C.b.* with *Bartonella quintana* and *Bartonella henselae* (La Scola *et al.*, 1996), *Legionella pneumophila* (Finidori *et al.*, 1992), *Legionella micdadei* (Musso and Raoult, 1997), and chlamydiae (Lukáčová *et al.*, 1999) may influence interpretation of results. To avoid this problem a cross-adsorption of serum samples should be done (Fournier *et al.*, 1998). It must be taken into consideration that the predictive value of positive and negative results can be affected by the disease prevalence in a given territory (McDade, 1991).

Coexistence of Q fever with other infections such as the Legionnaires' disease (Dobija-Domaradzki *et al.*, 1984) and Mediterranean spotted fever (Janbon *et al.*, 1989) may also complicate a correct and early diagnosis. In sera of patients with Q fever endocarditis, antibodies reactive with other agents than *C.b.*, e.g. *R. rickettsii*, *Ehrlichiae* and *Bartonella*, have been found (Singleton *et al.* (2002).

The immunoblot assay is also capable to distinguish acute from chronic Q fever (Blondeau *et al.*, 1990) by detection of proteins of high M_r (50 K, 80 K and 160 K). Antibodies to phase I *C.b.*antigens have been more common than those to phase II *C.b.* antigens. Several antigenic protein bands can be recognized only in sera from chronic Q fever patients.

A simple diagnostic method for detection of antibodies to *C.b.* is a sample collection method using blood samples dried on a blotting paper (Fenollar and Raoult, 1999). A dot blot assay can be used for identification of *C.b.* in tick hemocytes (Kocianová and Lukáčová, 1996).

Treatment

Acute Q fever in humans is a mild disease that can usually resolve spontaneously. However, because of the possibility of complications and chronicity, the patients should be treated with antibiotics. Tetracycline, administered in 500 mg four times a day, can reduce duration of the illness by 50%; it is effective provided the treatment started within 3 days after the first symptoms. At present, it is being replaced by doxycycline with two daily doses of 100 μg for 14–21 days (Spelman, 1982). Treatment of Q fever by this antibiotic is limited in patients with gastric intolerance and it is contraindicated during pregnancy or in childhood (Maurin and Raoult, 1999). Fluoroquinolones (ofloxacin and pefloxacin) are also very effective, mainly for the patients with Q fever meningoencephalitis, because they penetrate into the cerebrospinal fluid (Drancourt *et al.*, 1991). Macrolides as well as erythromycin (500 mg per day) may be used by children and pregnant women (Perez-del-Molino *et al.*, 1991). The *in vitro* erythromycin susceptibility varies among different strains of *C.b.* (Raoult *et al.*, 1991). This may explain resistance to the erythromycin treatment; hence

this antibiotic should not be considered a reliable alternative in Q fever therapy (Maurin and Raoult, 1999).

Treatment of chronic Q fever, especially endocarditis, is complicated by the ability of *C.b.* to resist the action of antibiotics. Therefore a long lasting therapy up to three years or until antibody titers fall bellow a border value is recommended (Raoult, 1993). However, Q fever endocarditis is still a severe disease with numerous relapses (Brouqui and Raoult, 2001). The treatment requires a multiple drugs approach. Various antibiotics and their combinations – doxycycline, rifampin, co-trimoxazole and clarithomycin (Maurin and Raoult, 1993; Marrie and Raoult, 1997; Siegman-Igra *et al.*, 1997) have been tested. A variable efficacy of quinolones, chloramphenicol and ceftriaxone has been demonstrated (Brouqui and Raoult, 2001). Acidic pH of lysosomes is likely to decrease the antirickettsial activity of some antibiotics such as tetracycline and its analogues (Yeaman *et al.*, 1989). pH of lysosomes can be increased by use of chloroquine, thus enhancing the antibacterial action of doxycycline (Maurin *et al.*,1992).

Tetracycline in combination with quinolone has been recommended as the first-line therapy of endocarditis for at least 4 years (Raoult *et al.*, 1999). Deoxycycline in combination with hydroxychloroquine applied for 1.5-3 years leads to fever relapses but requires examination of accumulation of hydroxychloroquine (Raoult *et al.*,1999). A successful two-years-treatment of endocarditis in a patient with biological prosthetic aortic valve and aortic homograft with a combination of dexycycline and chloroquine has been reported (Calza *et al.*, 2002). A recently recommended successful treatment (Maurin and Raoult, 1999) consists of a combination of doxycycline (100 mg twice a day) and chloroquine (200 mg three times a day) for at least 18 months or a combination of deoxycycline (the same dosage as above) and ofloxacin (200 mg three times a day) for at least 3 years. Besides the antibiotic therapy a valve replacement is often required, but it should be reserved for the cases with hemodynamic failure. After the surgery, to minimize potential infection, the antibiotic therapy should be continued for a long time (Maurin and Raoult, 1999).

Prophylaxis

Though Q fever occurrence has mostly an occupational character; a high contagiousness of *C.b.* and the possibility of developing chronic, sometimes fatal Q fever, indicate that professionally exposed individuals, mainly those handling livestocks and processors of animal products should be vaccinated. Another group of risk is veterinarians (Nowotny and Deutz, 2000; Abe *et al.*, 2001), veterinary technicians, students and personnel at schools of veterinary medicine and veterinary hospitals, and workers in cotton processing plants (Kazár *et al.*, 1982). Vaccination should be applied to persons at risk for chronic Q fever inclusive of those with cardiac valve defects, vascular aneurysmas and prostheses and immunocompromised patients.

Vaccines against Q fever have been developed and have successfully protected humans in Australia and former Czechoslovakia (Brezina *et al.*, 1974; Marmion *et al.*, 1990). Of importance is not only the choice of humans to be vaccinated but also the choice of the vaccine to be used (Kazár and Řeháček, 1987). Four different basic types of Q fever vaccines have been developed.

(i) Live attenuated vaccines M-44 and 1/m-44 were derived from the *C.b.* strain Grita in phase I of a highly reduced virulence but preserved immunogenicity (Genig *et al.*, 1965; Genig, 1968). However, biological properties of these vaccines were unstable and produced pathological changes such as myocarditis, hepatitis and splenitis in guinea pigs with the possibility of reactivation of the infection (Johnson *et al.*, 1977).

(ii) Highly purified corpuscular formalin-inactivated vaccine derived from the *C.b.* Henzerling strain prevented Q fever; no cases of the diseases after exposure to natural Q fever infection have been noted (Marmion *et al.*, 1984). This vaccine appeared to be safe and effective in preventing appearance of Q fever in abattoirs. The vaccine has been commercially available as "the Q-Vax vaccine" in Australia since 1989.

(iii) A soluble vaccine prepared from the *C.b.* strain Nine Mile corpuscles in phase I by extraction with trichloroacetic acid (Brezina *et al.*, 1974). It contains LPS and proteins.

It possesses endotoxin properties similar to those of Gram-negative bacteria (Schramek and Brezina, 1974). The protective nature of the vaccine was presumed from the fact that none of the laboratory personnel working with *C.b.* or the personnel working with *C.b.*-infected animals and vaccinated with the vaccine contracted Q fever (Kazár *et al.*, 1982; Marmion *et al.*, 1984; Ackland *et al.*, 1994).

(iv) In the USA, the use of a chloroform-methanol extraction residue subunit vaccine of *C.b.* strain Nine Mile in phase I of a low reactogenicity has been recommended (Fries *et al.*, 1993).

Though both the corpuscular and soluble vaccines derived from the *C.b.* in phase I are immunogenic enough, they are also reactogenic, causing post-vaccination reactions, mainly in previously infected individuals. Hence pre-vaccination serological and skin test examinations are necessary. The post-vaccination immune response can be evaluated by antibody response and cell-mediated immunity against *C.b.* antigens (Bell *et al.*, 1964; Ascher *et al.*, 1983; Worwick and Marmion, 1985). Antibodies to phase I or phase II *C.b.* antigens were detectable in about 50%–70% of vaccinees.

Vaccines for animals have also been developed. The vaccination has protected cattle against abortion (Behymer

et al., 1976), low fetal weight (Brooks *et al.*, 1986) and chronic infertility (Schmeer *et al.*, 1987). Inspite of protection, the vaccines did not eradicate *C.b.* from animals infected naturally prior vaccination, so that they could shed the agent to environment (Sádecký *et al.*, 1975b). Three types of animal vaccines have been developed.

(i) Inactivated whole *C.b.* corpuscle vaccine (Sádecký *et al.*, 1975a).

(ii) Chloroform-methanol extraction residue vaccine (Williams *et al.*, 1993; Kazár *et al.*, 1987).

(iii) Inactivated phase II corpuscle vaccine (Schmeer *et al.*, 1987).

The last vaccine has failed to protect goats against the *C.b.* infection (Fishbein and Raoult, 1992), thus confirming an earlier suggestion that an efficient animal Q fever vaccine should consist of or be prepared from the phase I *C.b.* (Kazár and Řeháček, 1987).

Despite existence of vaccines against animal Q fever, vaccination of animals is not widely used, because it is protective only in animals that are uninfected at the time of vaccination. To prevent the introduction and spread of *C.b.* it is necessary to stop an uncontrolled transport/migration of domestic animals into/within a country. Apart from preventive measures vaccination of domestic animals and humans at risk with currently available vaccines (Kazár and Brezina, 1991; Williams *et al.*, 1993) should be carried out. In Slovakia, the occurrence of Q fever in domestic animals and humans has been probably dramatically reduced due a large-scale vaccination of cattle in the 70-ties and 80-ties.

Significance for bioterrorism

C.b. is of significance for bioterrorism. Its high infectious potential (a single *C.b.* corpuscle may cause a disease in a susceptible host), resistance to heat and drying and easy spreading through humans by inhalation classifies *C.b.* as useful in biological warfare. Therefore it represents a potential terrorist threat (Kortepeter and Parker, 2000; Azad, 2002). As a matter of fact, *C.b.* was placed into the "Category B" of warfare agents, based on its threat for civilian population (Rotz *et al.*, 2002).

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