Coxiella burnetii – Pathogenic Agent of Q (Query) Fever

Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger»

1 Current Knowledge about the Pathogen

In 1937 in Queensland Australia, Derrick described a febrile disease, which he called ‘query fever’, in 20 of 800 workers of a meat factory in Brisbane [1]. He used the word ‘query’ because of the inexplicable nature of the disease. The pathogenic agent was isolated from the blood and urine of the patients in Australia by Burnet and Freeman and was called Rickettsia (R. burnetii) [2]. At the same time, the pathogen was isolated from ticks in Montana, USA, by Davis and Cox [3], and called R. diaporica, and, later on, renamed to Coxiella burnetii to honor both research groups.

The World Health Organization (WHO), the United Nations, and the Australian Group have classified C. burnetii as dangerous pathogenic agent [4]. The pathogenic agent was also an integral part of the biological arms program of the USA and the former Soviet Union, and is classified as Category B of pathogens with a potential for biological weapons by the Centers for Disease Control and Prevention (CDC) [5].

C. burnetii has a worldwide geographical distribution and can be transmitted to humans as zoonotic pathogenic agent [6]. According to the current state of knowledge, sheep, goats, and cattle are the main source of infection for man. In addition, cats, dogs, rabbits, wild animals, and ducks, as well as ticks and the feces of ticks, have been identified as sources of infection. The pathogen causes acute and chronic infections and is transmitted by contact or inhalation of dust aerosols and droplets, but can also be transmitted by consumption of raw milk and raw milk products [7, 8].

More than 30 genotypes of C. burnetii have been described, which can be distinguished by genome analysis [9]. Many of the genotypes have a worldwide geographical distribution, others are limited to a particular region. It has been established by means of pulsed field gel electrophoresis that some European and North American strains differ from each other [10], but that there are also strains which can be differentiated into regional groups such as German and Russian. The length of the genome of the bacterium is approximately 5 million base pairs (bp) [11].

1.1 Characteristics of the Pathogen

1.1.1 Structure

C. burnetii is a member of the family of the Coxiellaceae bacteria and replicates intracellularly in cells of different species. Phylogenetically related bacteria include Legionellaceae, Francisellaceae, Pseudomonaceae, and other Gammaproteobacteria. Coxiella are small Gram-negative, pleomorphic, coccoid bacteria with a size of 0.2–1.0 μm. They occur in 3 different forms: small cells (small cell variant, SCV) which are highly infectious, large cells (large cell variant, LCV) which develop also in cell culture, as well as spore-like particles (SLP) which are infectious and very robust to environmental conditions. Dependent on the host system, Coxiella undergoes a phase variation during growth [12]. In mammalian cells, bacteria grow as LCV, and form spore-like particles and 2 different antigenic forms described as Phase I and II.

Phase I

When C. burnetii replicates in cells of immunocompetent hosts, bacterial lipopolysaccharide (LPS) is synthesized in its full length, and additionally the cell wall antigens. C. burnetii is incorporated passively by the cells through phagocytosis and survives in the phagolysosome, also called parasitophorous vacuole, only at a low pH level, which is vital for the metabolic activity of the bacterium. Phase I Coxiella is extremely contagious for humans. Between 1 and 10 Coxiella form 1 human infectious dose (HID) and are sufficient for transmission of the infection [13]. The LPS is an essential virulence factor (cf. Section 1.2.). LPS is the main component on the surface of the external wall of the bacterium and covers the proteins. LPS thus blocks a rapid reaction of the immune system against the proteins of the bacterial wall. Coxiella LPS of the smooth type entails poor binding and activation of the components of the complement system, thus delaying or preventing lysis of bacterial cells [14]. The historical serologic distinction between the different Coxiella strains is based in the reactivity of LPS.
colors when stained using alkaline fuchsin and hematoxylin. distinguished morphologically, but they present different incompletely synthesized LPS. Phase I and II forms cannot be complement system. An intermediate form occurs with only virulence for humans, since they are rapidly inactivated via the low virulence in animal models and are considered as of little Coxiella and for diagnostic purposes. Phase II Coxiella show for the reaction of the immune system and characterization of pressed. Many cell wall proteins thus remain easily accessible additionally the production of some cell wall antigens is sup-
sion of the genes for LPS synthesis (raw type LPS of Coxiella); eggs), LPS is usually only incompletely synthesized by repres-
non-immunocompetent host system (e.g. embryonated chicken
forms of Coxiella are able to unfavorable external conditions. An overview of the different
The term ‘spore’ derives from environmental resistance. Spore-Like Particles
SLP was adopted from the term by morphologic similarities in electron microscopy with endospores of other bacteria spe-
SLP was adopted from the term by morphologic similarities in electron microscopy with endospores of other bacteria spe-
The 6 genogroups of C. burnetii and the MST genotypes assigned to these groups. Genogroup VI contains the so-called Dugway strain. The phylogenetic tree shows the close relationship of individual MST genotypes which have partly developed by convergent evolution. In this analy-
sysis, MST 14 and 15 are identical in Group II. Graphic modelled on Horn-
the spore-like particles (SLP). Both forms have a helical, long shape and 'fuss' are characteristic of Coxiella. Most of the environmental resistance is not comparable to that of bacillus or clostrid-
ion, many cell wall proteins are easily accessible for the reaction of the immune system and characterization of Coxiella and for diagnostic purposes. Phase II Coxiella show low virulence in animal models and are considered as of little virulence for humans, since they are rapidly inactivated via the complement system. An intermediate form occurs with only incompletely synthesized LPS. Phase I and II forms cannot be distinguished morphologically, but they present different colors when stained using alkaline fuchsin and hematoxylin.

Phase II

When C. burnetii is grown in culture cells or in cells of a non-immunocompetent host system (e.g. embryonated chicken eggs), LPS is usually only incompletely synthesized by repres-
sion of the genes for LPS synthesis (raw type LPS of Coxiella); additionally the production of some cell wall antigens is sup-
pressed. Many cell wall proteins thus remain easily accessible for the reaction of the immune system and characterization of Coxiella and for diagnostic purposes. Phase II Coxiella show low virulence in animal models and are considered as of little virulence for humans, since they are rapidly inactivated via the complement system. An intermediate form occurs with only incompletely synthesized LPS. Phase I and II forms cannot be distinguished morphologically, but they present different colors when stained using alkaline fuchsin and hematoxylin.

Spore-Like Particles

The term ‘spore’ derives from environmental resistance. Spore-Like Particles was adopted from the term by morphologic similarities in electron microscopy with endospores of other bacteria species, and is purely descriptive. The environmental resistance of Coxiella is not comparable with that of bacillus or clostridial spores. By the formation of SLPs, C. burnetii is able to remain infectious for more than 40 months even under very unfavorable external conditions. An overview of the different forms of C. burnetii can be found in the papers by Coleman et al. [15] and Angelakis und Raoult [11].

Plasmids

Four different plasmid types or chromosomally integrated plasmid-homologous sequences with a length of 36–56 kilo bp can be found in Coxiella of all phases [16]. Each bacterial cell has only 1 of the 4 plasmids, which contains around 2% of the genetic information [11, 17].

Genotypes/Genogroups

Based on the nucleic acid sequence analysis of different gene regions including the plasmids, a classification into genotypes or, if several genotypes were grouped together, geno-
groups, has been suggested by various authors. Individual genotypes display only partial agreement, but this agreement is sufficient to characterize outbreaks of disease – as occurred in England and the Netherlands – under molecular epidemiological aspects. Based on 10 selected regions, 173 Coxiella isolates from France were classified into 30 MST genotypes using the multispacer sequence typing (MST) method [9]. During the Q fever outbreak in the Netherlands, Coxiella isolates from Europe and other continents were classified into genotypes starting with the letters A to Q using ‘multiple-locus variable tandem repeat analysis’ (MLVA). I and J repre-
represented the dominant genotypes in cow’s milk and milk products [18]. I and J corresponded to the genotype MST-20 by Glazunova et al., 2005 [9] and were found in all goats, while earlier outbreaks in France and Germany were caused by MST-33 [19]. The latest outbreak in England was also caused by MST-20 [20]. Another study in the Netherlands which used the MVLA method found the A genotype as the predominant strain in 38 of 45 selected goat farms [21]. Finally, the MST grouping by Glazunova et al. [9] was extended by single nu-
cleotide polymorphism (SNP) analysis by means of real-time polymerase chain reaction (PCR) and melting point analysis considering insertions and deletions by bi-allelic and tri-allelic SNP [22]. By statistical analysis and creation of a phylogenetic tree, the Coxiella isolates could be classified into genogroups I–VI with individual groups containing between 1 and 15 MST (fig. 1). Part of the diversity was regarded as being due to mutations occurring in co-circulating Coxiella strains. MST geno-
types 14 and 15 by Glazunova et al. [9] were found to be identi-
tical in the genome analysis by Hornstra et al. [22]. Most of the MST genotypes have a worldwide geographical distribution. MST 8 causes chronic infections in humans, and frequently endocarditis. Goats act as the reservoir [22]. Patho-
genicity and virulence vary between the different genogroups [23].

1.1.2 Replication

In the infected organism, C. burnetii always replicates in-tracellularly, an extracellular replication in mammals has so far not been described. Host cells are cells of poikilothermic animals such as arthropods and fish, and cells of homeotherms such as birds, rodents, marsupials, and, in particular, domestic and livestock animals [24]. In placental cells of sheep, a patho-
gen number of 10^9/g placental tissue can be reached [7]. C. burnetii can survive in fresh water amoeba for several weeks [25]. The doubling period in mammalian cells is 20–45 h [11].
After transmission, *C. burnetii* is phagocytosed by monocytes and macrophages in humans, and transported into the phagolysosome. There, a low pH level is created by Coxiella, thus preventing the decomposition of the bacterium; subsequently the replication process is started. Besides the normal bacterium, the SLP is generated with a thickened wall which is metabolically inactive [12] and is thus not affected by antibiotic treatment (cf. Section 3.4).

*C. burnetii* intracellularly suppresses the production of oxygen derivatives and NO, synthesis of interferon gamma, and the formation of suppressor T-lymphocytes. The migration of immune defense cells to the replication sites during the acute phase causes the granulomas that are typical for the chronic disease [26] (cf. Section 1.2.2.).

In the past few years, studies have been performed on the replication of *C. burnetii* in cell-free cultures. The pathogenic agent was considered as prototype of a bacterium with the requirement of intracellular growth in eukaryotic cells. In infected cells, *C. burnetii* replicates in the acidic environment of the parasitophorous vacuole. More recent studies show that *C. burnetii* can replicate in special media in cell-free cultures [27, 28]. This observation opens new pathways for a targeted investigation of pathogenicity mechanisms and the development of therapeutic intervention strategies.

### 1.1.3. Susceptibility to Inactivation and Stability under Environmental Conditions

*C. burnetii* SCV is very stable to environmental conditions and can remain infectious for many months [29] (cf. Section 1.1.1.). Phase I and II forms are destroyed by 2% formaldehyde, however, infection-competent *C. burnetii* could be extracted after 4–5 months from formalin-fixed tissue. Additionally, replication-competent *C. burnetii* was claimed to be isolated from paraffin-treated tissue. Likewise, the sterilization process can be inefficient using gaseous formaldehyde [29]. Effective inactivation of Coxiella is achieved by 1% phenol, 5% hydrogen peroxide, 5% chloroform, 0.5% hypochlorite, and exposure to heat at 65 °C and above for 1 h [30, 31]. Irradiation with 10 kGy gamma rays inactivates Coxiella completely, while its antigenicity is preserved [32]. Disinfection is possible using 70% ethanol for 20 min. Autoclaving at 131 °C for 15 min inactivates *C. burnetii*; as well treatment using 5% formaldehyde for 5 min. The bacterium survives as spore stage (SLP) on wool of sheep for 7–10 months at 15–20 °C, for more than 1 month at 4 °C on fresh meat, and for more than 40 months in dry milk powder at room temperature [33, 34].

### 1.2 Infection and Infectious Disease

As mentioned above, *C. burnetii* is extremely infectious for humans, 1–10 viable organisms suffice to induce an infection via the aerogenic route [35]. In hospitalized patients in France, the mortality rate was 2.4% [31].

Coxiella LPS is not toxic for hen embryos, even in very high doses [36]. Infected animals such as sheep and goats show no signs of disease [37]. Pregnant domestic animals may abort following an infection with *C. burnetii* [38]. In humans, distinctions are made between acute and chronic Q fever, and depending on the symptoms and clinical signs, between pneumonia, endocarditis, hepatitis, and neurological manifestations.

#### 1.2.1 Acute Disease

At least 50% of the human infections with *C. burnetii* are asymptomatic. After an incubation period of 3–30 days, unspecific symptoms such as fever, sweating, nausea, vomiting, and diarrhea occur, and extreme fatigue in 5–20% of the patients. Frequently headache may occur which does not improve after administration of analgesics. The bacterium can be present in the blood during this acute stage. The disease is often self-limiting [39]. In endemic regions such as southern Spain, the Basque region and France, fever lasting more than 1 and less than 3 weeks is typical for Q fever [40].

Pneumonia occurs in 1–2% of acutely affected patients. The most frequent form of the disease is lobar pneumonia with high fever. Furthermore, the disease manifests itself as atypical pneumonia and rapidly progressing pneumonia. This form of pneumonia often occurs in connection with sharply delimited lung infiltrates on X-ray and consolidation of the alveolar cavity. Infiltrates can be present segmentally and non-segmentally. The rapidly progressing pneumonia clinically resembles the pneumonia caused by *Legionella pneumophila*. Atelactasis and swelling of hiliar lymph nodes can occur. X-ray shows the resulting granulomas as multiple round opacities. Acute pneumonia may progress to the chronic form [41]. 5% of affected individuals have concurrent splenomegaly, and, during this period, *C. burnetii* can also be isolated from the cerebrospinal fluid [42].

#### 1.2.2 Chronic Disease

Around 5–15% of acute cases become chronic. Manifestations include endocarditis, vasculitis, osteomyelitis, hepatitis, interstitial lung fibrosis, and long-lasting or recurring fever. An analysis of the > 4,000 cases of Q fever in the Netherlands showed the following risk factors for chronic course: preliminary cardiac valve surgery, blood vessel prosthesis implants, aneurysm, renal insufficiency, and advanced age [43].

*Endocarditis*

The entire vascular system can be affected, above all, artificial valves and the walls of aneurysms, on which *C. burnetii* adheres as biofilm. Endocarditis is the major manifestation of Q fever and comprises around 10% of all cases of endocarditis in England and Wales [44]. Endocarditis patients frequently present with splenomegaly; arterial embolism can also occur. Endocarditis is associated with anemia and hematuria. Endocarditis caused by Coxiella very rarely occurs in children. In 53% of the cases of endocarditis caused by *C. bur-
netii, the bacterium can be detected in blood. The bacterial load rapidly decreases if doxycycline and chloroquine are administered. If antibiotic treatment is transiently interrupted, Coxiella resistant strains are selected within 3 years and lead to relapses of endocarditis [45, 46]. Coxiella survives for years in granulomas which are formed in various organs, not only restricted to heart and cardiac valves tissues [46].

Hepatitis

While hepatitis caused by C. burnetii is very rare in the USA and in England, it is the most frequent manifestation of chronic C. burnetii infection in France and Spain in regions of intensive sheep breeding [31]. Consumption of raw milk and cheese from raw milk seems to be associated with hepatitis development. Hepatitis can be confirmed clinically and by laboratory tests (e.g. elevation of alanine transaminase) with formation of granulomas in the liver biopsy sample histology. The granuloma has a dense fibrin ring [47]. Hepatitis can be an additional manifestation found in patients with Q fever pneumonia, or Coxiella may damage only the liver. Hepatitis caused by Coxiella with elevation of transaminases may present without an increase in inflammatory parameters such as C-reactive protein [48].

Neurological Manifestation

Symptoms caused by Q fever include severe headache, aseptic meningitis, and encephalitis. In the cerebrospinal fluid partly mononuclear cells are found; the protein content is increased, and C. burnetii may be isolated. Meningitis, reduced vision, paresthesia, and impaired sensory abilities can occur. Guillain-Barré syndrome caused by C. burnetii has been described in the literature [7]. In the eye, Coxiella can cause uveitis and retinal detachment [49]. 20–30% of affected individuals complain of chronic fatigue syndrome as post-fever manifestation. During this period, Coxiella can be identified partially, or periodically, via the DNA-NAT (nucleic acid amplification test) in blood and bone marrow [13, 50, 51].

Infection in Domestic Animals

Infections with C. burnetii in domestic animals are usually asymptomatic. During the acute phase, the pathogen can be detected in blood and in organs such as lung, liver, and spleen. In the chronic phase, C. burnetii is excreted in the feces and urine but also in the milk. Pregnant animals may suffer miscarriages (sheep and goat) and produce offspring with low birth weight (cattle). High amounts of Coxiella can be found in birth products such as the placenta and in the newborn animal [52, 53].

1.3 Epidemiology

Q fever caused by C. burnetii is a zoonotic worldwide distributed infectious disease. In Europe, it reaches its seasonal peak in spring and early summer [54]. Ticks infected with C. burnetii seem to play an essential role in maintaining the transmission cycle in the natural environment of wild-type animal species. Ticks excrete high quantities of infectious pathogens in the feces, thus being able to spread Coxiella. During the outbreaks in livestock, the close contact among the animals seems to play the most essential role in the transmission of the pathogenic agent [11].

Direct and indirect detection of infection with C. burnetii is notifiable giving the name of the infected person pursuant to the German Infection Protection Act (Infectionsschutz-Gesetz (IfSG) Section 7, (9)). The annual incidence in Germany is around 1–5 cases per 10^6 inhabitants. In the period from 2000 to 2011, between 200 and 500 infections were reported to the Robert Koch-Institute annually. The majority of the infections occurred regionally in clusters and was related to farming or having contact with sheep [55], particularly during lamming [54]. A major outbreak of Q fever could be related to lamming on a farm market at which 300 persons developed the disease [56].

During the epidemic in the Netherlands of 2007–2009, C. burnetii was above all transmitted by goats and goat dung [57]. At the center of the epidemic (Hertogenbosch), 10.7% (84 of 785) of the persons tested in the screening program had Phase II IgG antibodies, resulting in an estimate of around 40,600 exposed persons for the entire region [58]. In Europe, outbreaks of Q fever in sheep herds occurred in the German Federal states of Baden-Württemberg [59], Bavaria [60] and Thuringia [61], as well as in France [62], Cheltenham in England [63], and Bosnia [64]. C. burnetii infected individuals were also identified among soldiers returning from Kuwait [65], Iraq [66], and Afghanistan [67].

Animals infected by C. burnetii endemically without any signs of disease include ruminants such as sheep, goats and cattle, but also dogs, rabbits, cats and birds [11] (cf. Section 1.). The reservoir for renewed infestation of endemic areas after culling potentially infected livestock (goats and cattle) and sterilization of contaminated manure in the Netherlands was most probably rats of the Rattus norvegicus and Rattus rattus species, in which a prevalence of Coxiella DNA in the spleen of 3–5% and an antibody prevalence in the serum of 14–50% was found [68].

Transmission Routes of C. burnetii

Aerogenic: inhalation of contaminated aerosol or dust [63, 65, 69–72]. This is the main transmission pathway for humans [73]. The risk of Coxiella transmission by dust persists even years after the sheep herds have left the region [74].

Contact: Human to human, animal to human, tick fecs to human; direct and indirect contact with contaminated tissue, such as sheep placenta or goat manure [75]. Tick fecs can be highly infectious, since one gram of tick fecs contains around 10^7 Coxiella. After lamming, infectious Coxiella can be detected in the soil for up to 150 days [33]. C. burnetii can be transmitted by exposure to contaminated straw, enclosures, or
Direct non-sexual transmission between humans has so far not been established [90]. However, a possible transmission between humans who are members of the same household has been discussed [91]. *C. burnetii* has been transmitted during autopsy. Only 1 transmission by blood transfusion is known [92–94]. Laboratory infections with *C. burnetii* have occurred repeatedly [30, 95], therefore safety level 3 conditions are required for the replication of Coxiella. Laboratory infections with *C. burnetii* rank 4th of all lab infections in England. In conformity with the current regulations governing biological materials, Coxiella is classified as biosafety level 3.

Cattle, sheep, and goats are regarded as the main source of infection with *C. burnetii* for humans. In the past few decades, studies have been conducted on the distribution of *C. burnetii* infections in livestock. Guatteo et al. [96] reported a prevalence of *C. burnetii* infections in dairy farm animals of 20–38% in cows and 15–25% in sheep and goats. These studies were typically conducted in restricted regions only and on a relatively small number of animals, so that the figures provide only a rough estimate of the prevalence in the various regions. For Germany, more recent studies are available from the federal states of Saxony and Thuringia, which show that antibodies against Coxiella can be detected in some herds [61, 97].

**1.4 Detection Methods**

**1.4.1 Serological Detection Methods**

*Antibodies:* Besides IgM and IgG, also IgA antibodies against *C. burnetii* can be detected. Antibodies become detectable 2–3 weeks after occurrence of clinical symptoms. They then usually increase for some months and persist for years [fig. 2]. According to some authors [98], the cross-reactivity of *C. burnetii* antibodies with other bacteria is low, however, it cannot be ruled out with some antigens of legionella, bartonella, and pseudomonas [99–101]. High antibody titers against Phase I, which barely decrease over a period of 600 days, point to a chronic course [45, 102, 103]. IgM Phase II is still detectable 12 months after transmission of Coxiella in 62–83% of the blood samples [104].

**Fig. 2.** Temporal course of *C. burnetii* infection and serological response, detectable through Phase I and II antibodies. In the chronic course, DNA can be detected periodically after acute infection, and antibody titers remain high. Diagram based on Van Wijk et al., 2011 [134] (L. Guertler).
Indirect immunofluorescence assay: The micro-immunofluorescence assay is considered as the reference standard. Its set-up was described as early as 1983 [105]. The assay becomes positive around 1–2 weeks after appearance of the symptoms, additionally IgM antibodies can be detected using this method [106]. Typically, titers of ≥ 1:800 are considered positive for IgG for chronic Q fever [107], and titers of ≥ 1:50 for IgM and ≥ 1:200 for IgG [108]. The evaluation should be performed according to the titer values provided by the manufacturer of the test. As usual, seroconversion or titer levels that increase 4-fold are regarded as positive for the detection of an acute or reactivated infection.

ELISA, agglutination test, and complement-fixation assay (CFA): All 3 tests have been developed for routine diagnostics, CFA was most frequently used for early prevalence studies [109]. CFA is regarded as positive if titer against Phase I are > 1:200 during chronic Q fever. Antibodies against Phase II antigens are considered as positive at ≥ 1:40 during acute Q fever. Because of the high workload and the non-detectability of IgG2, IgG4 and IgA, as well as lacking standardization, CFA is nowadays replaced by ELISA or micro-immunofluorescence tests. Only a few manufacturers offer ELISA for the detection of C. burnetii antibodies. Phase I and II, and IgM and IgG are determined in separate assays. ELISA was used for the identification of infected blood donors in the Netherlands, and reactive samples were further analyzed by PCR [102, 104].

Western blot: 15 antigens are present on the strip for C. burnetii grown in Phase 1, with a molecular weight of 20–160 kD. 7–10 different antigens may be stained with sera from the acute infection, especially the bands with 50, 80 and 160 kD; while 12–15 antigens may be stained with sera from the chronic phase [110, 111]. If the C. burnetii infection is treated with antibiotics, a slow decrease in antibody titers takes place. IgM antibodies can be detectable over a period of 670 days [112]. In another study, IgM antibodies could still be detected in 3% of 162 individuals after 1 year [113]. IgA antibodies are detectable in higher titers in chronically C. burnetii-infected individuals [106].

1.4.2 Direct Detection of Coxiella burnetii

1.4.2.1 Culture

The detection of C. burnetii in cell culture is performed on L929 fibroblasts or other cells such as vero cells [27, 114, 115]. For analysis, samples are extracted after filtration using 0.45 μm filters. Suitable for analysis are extracts from tissue samples and blood before the initiation of antibiotic treatment [116]. Culturing C. burnetii must be performed under safety level 3 conditions to avoid the risk of laboratory contamination [95]. More recent studies show that Coxiella can be cultured in acidified citrate cysteine (ACC) medium as Phase II Coxiella after an incubation period of around 6 days [27, 117]. The bacteria grew by up to 3 log10 and were infectious [27]. Since only few laboratories have an authorization for handling replication-competent biosafety level 3 pathogens, detection and quantification of Coxiella, if required, is usually performed by PCR. If SCID mice (combined immunodeficiency disease) are inoculated with Coxiella-contaminated material, 1–10 bacteria can be detected [115, 117].

1.4.2.2 NAT – Nucleic Acid Amplification Test

Conventional PCR, nested PCR, or real-time PCR are used for the detection of C. burnetii nucleic acid [118]. Primers used are derived from the superoxide dismutase gene [119], plasmid or other gene segments [9, 120]. In addition, Coxiella DNA can be successfully detected in paraffin-embedded tissue. Real-time PCR with a sensitivity of 1 copy DNA has been developed [121]. Coxiella can be detected in the blood by PCR 2 weeks after transmission [104]. Further frequently used primer-binding sites for PCR include genome regions with repetitive elements and the outer membrane protein OMP [122–124]. Reliable PCR detection methods for Coxiella have been used for genotyping (see Section 1.1.) [9, 18, 22]. A non-symmetrical PCR with hybridizing and analysis of the products in solution, which simultaneously detects further bacteria, has been developed in the Netherlands [125]. The most extensive characterization of Coxiella strains was performed by amplification and sequencing of 139 chromosomal and plasmidic open reading frames (ORFs), including the genes for LPS biosynthesis [120]. This method was used for characterization of the strains of the outbreak in the Netherlands [20] and the strains in Spain [126].

2 Blood and Plasma Donors

2.1 Prevalence and Incidence in Donor Collectives

Up to now, no transfusion-associated C. burnetii transmission has been reported in Germany. Table 1 lists the prevalences of Coxiella antibody in blood donors of several countries [14]. Blood donations in Germany are currently not tested for the presence of C. burnetii, or antibodies against C. burnetii. C. burnetii is present also in Germany in areas with a high density of sheep. How efficient infected donors are excluded from donating blood based on febrile symptoms and the general fatigue associated with the infection depends on the symptoms present at the time of donation, the recording of anamnesis, and on whether or not local outbreaks become known in time. 60% of primary infections in adults show an asymptomatic course [11]; according to data from the Netherlands [127], the bacteremia which occurs during this phase is in the range of a few per mille [128]. Generally it should be assumed that asymptomatic individuals will donate blood, partially during the acute, and partially during the chronic phase of the disease [93]. Since C. burnetii grows in macrophages and monocytes, the bacterium can rapidly be spread by the bloodstream of the body in these cells. In persons with chronic infestation of the blood vessel walls, it should be expected that C. burnetii is temporarily released. In persons with acute infection, C. burnetii is spread via the blood and lymph system so that the bacterium can be detected in cer-
only by using ELISA [128]. Routine antibody testing is not considered necessary based on the current epidemiological data in Germany. The detection of Coxiella antibody in general indicates immunity, not infectivity. However, increased IgM and IgG titers were found in 38 of 942 blood donors tested in the South of France; 2 of these 38 donors (total 2/942, 0.2%) developed acute Q fever which was undetectable at the time of the donation [31].

2.3.2 Detection of Coxiella burnetii

The very time-consuming culture under biosafety level 3 conditions in the shell-vial test (e.g. 48-well microtiter plates) is unsuitable as a screening test. Detection via DNA as NAT, e.g. as PCR, is not routinely performed and, considering the current epidemiological situation, not necessary. In blood donors in the Netherlands, 1,004 donors in the area with the highest C. burnetii incidence were tested using PCR, and 3 (0.3%) were identified as being genome-positive [134]. The strategy shows that NAT can be introduced if required. It is unclear to what extent PCR-positive donors can infect recipients, since up to now only 1 Coxiella transmission by blood transfusion has been reported worldwide [93]. In human blood, leucocyte-depleted blood, red blood cell concentrate, and plasma spiked with C. burnetii the pathogenic agent remained infective for more than 6 weeks [135]. The bacterium could only be removed from rat serum spiked with C. burnetii by filtration with 0.1 µm filters, while heat treatment at 56 °C for 30 min was ineffective [136]. For Germany, there is a need for research to elucidate the importance of exposure to bacteremia, the type and duration of infectious chains, and analysis of the circulation of various Coxiella strains.

2.4 Donor Interview

Interviewing donors specifically concerning the possible risk of acquiring C. burnetii infection seems to be indicated only if a Q fever outbreak has been announced. Usually animals infected with C. burnetii show no symptoms of the disease, however, may excrete the pathogen in feces, urine, vaginal secretion, and milk over longer periods, so that a donor interview regarding contact with domestic animals or live-

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Seroprevalence, %</th>
<th>Author [ref.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Netherlands</td>
<td>1987</td>
<td>45</td>
<td>Richardus et al. [127]</td>
</tr>
<tr>
<td>France</td>
<td>1992</td>
<td>4</td>
<td>Tissot-Dupont et al. [31]</td>
</tr>
<tr>
<td>Newfoundland</td>
<td>2001</td>
<td>8</td>
<td>Hatchette et al. [129]</td>
</tr>
<tr>
<td>Germany</td>
<td>2001</td>
<td>15–22</td>
<td>Hellenbrand et al. [54]</td>
</tr>
<tr>
<td>Spain</td>
<td>2007</td>
<td>23 (IgG), 0.3 (IgM)</td>
<td>Bartolomé et al. [131]</td>
</tr>
<tr>
<td>Turkey</td>
<td>2008</td>
<td>32 (IgG), 3 (IgM)</td>
<td>Kılıç et al. [130]</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>2012</td>
<td>12 (IgG)</td>
<td>Hogema et al.* [128]</td>
</tr>
</tbody>
</table>

*False-positive reactions in the test collectives cannot be ruled out.

Table 1. Seroprevalence of C. burnetii among blood donors

ebospinal fluid and urine [132]. The detectability of Coxiella in various body fluids supports the data that C. burnetii can cause temporary bacteremia.

2.2 Definition of Exclusion Criteria

General exclusion criteria, which might indicate an acute or chronic infection, apply to the deferral of blood donors possibly infected with C. burnetii, such as elevated body temperature, weakness, fatigue, diarrhea within 1 week before the donation, and anemia. Concerning regional outbreaks, known risk factors include contact with sheep, proximity and duration of stay at a potential transmission site, as well as exposure to contaminated animal material and dust within a radius of 5 km (cf. Section 1.3). Also short episodes of fever after contact with a Q fever sufferer are a possible exclusion criterion. The final deferral from the donation is decided dependent on the epidemiological situation and the individual risk assessment by the doctor. When asymptomatic individuals might have been exposed to C. burnetii, a lag period of 4 weeks should pass before the next donation. If a Q fever infection had happened that was cured with or without treatment with doxycycline plus chloroquine, the donor can be readmitted to donation 4 months after the symptoms have disappeared [133]. If blood was donated during the incubation period of C. burnetii, the blood components prepared from that donation must be recalled according to the known risk situation; these blood components should not be used for transfusion (exceptions, see Section 3.6). If the blood product was already transfused, treatment of the recipient with doxycycline plus chloroquine or fluoroquinolone should be considered depending on the clinical symptoms of the recipient. Further follow-up activities analogous to the look-back procedure for viral infections (Vote 34 of the German Advisory Committee ‘Blood’ (Arbeitskreis Blut)) are not required.

2.3 Donor Testing and Significance

2.3. Detection of Antibodies

Detection of C. burnetii antibodies in blood donors is not performed; when needed for technical reasons it is possible
stock is indicated only when an animal infection with *C. burnetii* has been confirmed. Clinical symptoms of acute *Coxiella* infection, e.g. flu-like symptoms, or of a chronic infection, e.g. relapsing endocarditis, are covered in the general donor interview, and individuals with a suspected infection are excluded from blood donation.

2.5 Donor Information and Counselling

Information and counselling specifically focused on *C. burnetii* will become necessary at a regional level if an outbreak has become known. The risk of transmitting an infection is imminent predominantly at farm markets during lamming, at animal fairs, after close contact with manure, during and after passing by sheep herds, after inhaling contaminated dust, and during processing natural wool. Since transmission of *C. burnetii* can occur by dust and wind, the regional influence of weather conditions must also be considered when assessing the infection risk of donors during local outbreaks [63, 65, 73]. Q fever can also be acquired by travelling to endemic countries as was shown in the case of a traveler returning from Australia to the USA and 5 travelers returning from Africa, South America, and the Philippines to Spain [137, 138]. When needed, donor counselling and interviewing is carried out focusing on the specific symptoms of Q fever (as described in Section 1.2).

3 Recipients

3.1 Prevalence and Incidence of Blood-Associated Infection and Infectious Disease in Recipient Populations

Few data are available on assessing the Coxiella prevalence and incidence of recipients in Germany. It should be assumed that immunity differs depending on the region. For rural areas, Coxiella antibody prevalences of up to 7% were reported from France [101], 11–17% from Switzerland [69], and 4% among non-exposed and 15% among exposed individuals in England [139]. Around 12% of the population of Montana, USA, have *C. burnetii* antibodies [140]. A Coxiella antibody prevalence of 38% (162/424) was found among veterinarians in Germany [60].

3.2 Immune Status (Resistance, Existing Immunity, Immune Response, Age, Exogenous Factors)

A natural resistance against *C. burnetii* does not exist in humans. A small number of bacteria can be inactivated by complement lysis. Immunity is built up after recovery from acute infection; however, some infections proceed to assume a chronic course as is evident in patients with endocarditis who partially develop insufficient immunity and thus persistent infection (see Section 1.2.2.). An immune response can be detected using specific antibody tests 2–3 weeks after infection (see Section 1.4.1.). At a more advanced age, the proportion of occurring complications following acute *C. burnetii* infection is increased. Factors that aid chronic *C. burnetii* infection include immune deficiency, e.g. caused by infections such as HIV [101] or immunosuppressive treatment. Chronic *C. burnetii* infections are found at a higher rate in immunocompromised individuals [100].

3.2.1 Vaccination

A vaccine against *C. burnetii* has been produced by growing of the bacterium on hen’s egg yolk sac, enrichment, and subsequent formalin inactivation [141]. However, up to 1994, this vaccine was not used extensively in both risk groups with contact to sheep and in sheep livestock, even though none of the vaccinated individuals in Australia developed Q fever [142]. An additional vaccination program was developed successfully in Australia in 2008, designed to reduce the risk of Q fever infection in persons with an infection risk such as workers in abattoirs or sheep farms [143]. In the Netherlands, health authorities also recommended vaccinating individuals at risk of severe illness following *C. burnetii* infection [57, 144]. It remains unclear whether vaccination against Q fever in Germany is useful for individuals at risk of developing a severe *C. burnetii* infection or at high transmission risk. The answer to this question also depends on the future epidemiology of *C. burnetii*.

A commercial inactivated Coxiella Phase I vaccine manufactured in Hungary has been available for immunizing animals since 2010. Used in sheep, the vaccination initially did not prevent infection but lead to a reduction in the excretion of Coxiella by 92–98% [37]. The vaccination was most effective if administered during an animal’s first pregnancy [57]. After vaccination, Coxiella DNA of the vaccine was found in the milk of vaccinated goats within a few hours to 9 days [79]. 2 years after vaccination, the sheep and their offspring no longer excreted Coxiella, however, even after 4 years, Coxiella was still detectable in the animals’ environment [145], which underlines the significance of animal reservoirs such as rats [68]. It is yet unclear, according to current knowledge, to what extent vaccinating livestock such as cattle, sheep, and goats influences the epidemiology of *C. burnetii* [146]. Although vaccinating sheep and goat herds was introduced in the Netherlands during the *C. burnetii* epidemic in 2009, the extent to which this measure has contributed to reducing the incidence of the disease remains to be seen [144].

3.3 Severity and Course of the Disease

The majority of all Coxiella infections (> 60%) with or without fever are self-limiting [39]. In the event of clinical manifestation, a 2% death rate should be expected [31] depending on the time of initiation of antibiotic treatment, age,
and immune status of the infected individual. As described in Section 1.2, infection with \textit{C. burnetii} can be asymptomatic, or acute, or progress to a chronic outcome.

### 3.4 Therapy and Prophylaxis

Doxycycline is the preferable treatment. Other effective treatments include ampicillin, chloramphenicol, fluoroquinolones, and, in some instances rifampicin in combination with doxycycline or ciprofloxacin [147]. Antibiotics such as erythromycin, gentamicin, penicillin G, and streptomycin are not effective [148]. Acute infections should be treated for at least 2 weeks. Chronic infection is treated until symptoms subside, or for 3 years preferably with doxycycline in combination with hydroxychloroquine, in order to alkalize the contents of the phagosome, or with ampicillin. Some authors recommend lifelong treatment in the case of endocarditis [112] since repeated reactivations (relapses) have been observed during treatment interruptions [45]. More recent alternatives for antibiotic treatment include tigecycline and clarithromycin [114].

### 3.5 Transmissibility

As described in Section 1.3., \textit{C. burnetii} is highly contagious; 1–10 bacteria suffice to infect a human. Transmission occurs most frequently via inhaled or ingested contaminated material. In placental tissue of infected animals, concentrations of up to 10^9 bacteria per gram of tissue can be reached [7]. Intrauterine transmission has been described in humans [88]. The spore-like particles as permanent forms of \textit{C. burnetii} remain infectious in the environment for many months, so that the end of transmissibility after an outbreak of the disease is difficult to predict, and transmissibility can last several years [145]. Transmission by blood is possible theoretically, however, only 1 case has as yet been described [93]. A further possible case has been discussed in the Netherlands, however, the recipient originated from a region which was highly affected by the Dutch Q fever epidemic [128, 144]. Transmissions from human to human can occur within families by contact with contaminated laundry [78]. During patient treatment, no nosocomial transmission was observed [13]. Sexual transmission during the acute infection phase is described in Section 1.3 [83, 84].

### 3.6 Frequency of Administration, Type, and Amount of Blood Products

Transmission of \textit{C. burnetii} by blood and blood products has so far not been reported in Germany. Coxiella transmission by fresh frozen plasma is theoretically possible but has not yet been reported. The risk of a transmission by plasma products can be ruled out since bacteria are removed during fractionation and filtration, and are inactivated or destroyed during heat or solvent-detergent treatment of the products.

### 4 Blood Products

#### 4.1 Infectious Load of the Starting Material and Test Methods

The infectious load in blood and plasma is unknown in Germany but must be considered as being very low. In 863 blood donors in Albacete, South-East Spain, the seroprevalence of anti-phase II IgG was 23.1%, and that of IgM, indicating a possible acute infection, 0.3% [131]. Among 1,004 blood donor samples in the Netherlands in 2009, 3 (0.3%) contained Coxiella DNA, while Coxiella IgG could be detected in 66 of 543 (12.2%) of the donors [128]. 10 donors seroconverted during the investigation period, which corresponds to an incidence of 5.7% per year [128]. \textit{C. burnetii} cannot be removed from blood and cellular blood components since the bacterium is present intracellularly, i.e. it is cell-associated and also grows in monocytes. During acute infection, \textit{C. burnetii} is also present in granulocytes, however, cannot be removed totally by leucocyte depletion. During the Q fever outbreak in the Netherlands, a specific donor selection strategy was developed to reduce efficiently the risk of Coxiella transmission: Exclusion of all donors who have a professional risk of exposure, all donors who live within a radius of 5 km from the center of the outbreak, and all donors who show flu-like symptoms or had such symptoms within the past 2 weeks; and additional testing of all donations with a phase I IgM ELISA, even though a high false-positive rate was expected using this test [134]. Testing blood donors for phase I IgG has a 100% sensitivity only if the titer is above 1,024 [144]. By use of this exclusion strategy, antibody test, and donor selection, no evidence for Coxiella transmission by transfusion was found in the Netherlands [144, 149].

#### 4.2 Methods of Removal and Inactivation of the Infectious Agent

##### 4.2.1 Removal

Derichment of \textit{C. burnetii} can be performed by leukocyte depletion, however, this procedure will be incomplete. Derichment from plasma can be performed by high-speed centrifugation or filtration through a 0.2-\(\mu\)m filter. Small particles of \textit{C. burnetii} can, however, also pass through the membrane of 0.2 \(\mu\)m filters.

##### 4.2.2 Inactivation

Studies on the inactivation of \textit{C. burnetii} by heat treatment (pasteurization) are available only for milk products [150]. It
can be assumed that inactivation of the large cell variant by plasma pasteurization (60 °C, 10 h) is possible, and the small cell variant should be inactivated in a time-dependent manner. Trials for the thermo-inactivation of the variants have not yet been published.

4.3 Feasibility and Validation of Procedures for Removal/Inactivation of the Infectious Agent

_C. burnetii_ can be replicated in cell culture at high concentrations. Culturing requires biosafety level 3 conditions. Theoretically, the efficiency of pathogen reduction and inactivation can be tested by spiking. However, it is not necessary to validate the inactivation as long as the epidemiological situation in Germany remains constant, since until now no _C. burnetii_ infections by blood and plasma products have occurred in Germany.

5 Assessment

_C. burnetii_ is a bacterium which occurs in many animal species worldwide. _C. burnetii_ can remain infectious in soil and environment as spore-like particles for many months. Various _C. burnetii_ strains show different degrees of pathogenicity. The hazard of the infection is its chronic course with endocardial and vascular lesions, granulomatous hepatitis, and pulmonary fibrosis. Intratracheal transmission can also occur in humans.

The incidence of _C. burnetii_ is related to exposure to contaminated dust and contact with infected animals. Outbreaks in rural regions in Germany and other European countries with intensive sheep and goat farming have so far not led to transmission of the bacterium by blood donation. The risk of transmission of _C. burnetii_ by transfusion is thus very small. Likewise, no transmission of _C. burnetii_ by plasma or plasma products has been reported.

A preventive deferral of donors originating or returning from regions with _C. burnetii_ outbreaks for 4 weeks after the last human case has been reported is a suitable measure to reduce the infection risk [51, 151, 152]. The hygienic measures implemented in the Netherlands during the _C. burnetii_ outbreak of 2007–2009 have contributed to preventing _C. burnetii_ transmission by blood transfusion [128]. As a risk reduction measure, no arrangements for blood donations should be fixed during the recommended 4-week period in areas with increased risk of _C. burnetii_ exposure. Local outbreaks should therefore be announced promptly. In the case of a massive local outbreak, during which donor deferral could lead to shortages in blood supply, NAT testing could alternatively be considered to reduce the risk of _C. burnetii_ transmission.

The risk of _C. burnetii_ transmission by blood components is extremely low so that initiating a look-back procedure as defined for viruses in Votum 34 of the Advisory Committee ‘Blood’ (Arbeitskreis Blut) following a local _C. burnetii_ outbreak after the collection of blood donations does not seem beneficial.

This paper was completed on 26 October 2012 and approved by the German Advisory Committee ‘Blood’ (Arbeitskreis Blut) on March 5, 2013. It was compiled by the members of the subgroup ‘Assessment of Pathogens Transmissible by Blood’ of the German Advisory Committee ‘Blood’ (Arbeitskreis Blut): Prof. Dr. Latz Gürttler, Dr. Ursula Bauerfeind, Dr. Johannes Blümel, Prof. Dr. Reinhard Burger, Prof. Dr. Christian Drosten, Dr. Albrecht Gröner, Dr. Margarethe Heiden, Prof. Dr. Martin Hildebrandt, Prof. Dr. Dr. Bernd Jansen, Dr. Ruth Offergeld, Prof. Dr. Georg Pauli, Prof. Dr. Rainer Seitz, Dr. Uwe Schlenkrich, Dr. Volkmart Schottstedt, Dr. Johanna Strobel, Dr. Hannelore Willkommen.

References

Arbeitskreis Blut

Transfus Med Hemother 2014;41:60–72

Coxiella burnetii – Pathogenic Agent of Q (Query) Fever

Transnat Med Homoter 2014;41:60–72


AFSSPS – Agence française de sécurité sanitaire des produits de santé. Risque de transmission de *Coxiella burnetii* par les produits de santé, November 15 2002.


