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Arbobacteria – Pathogens Transmittable by Arthropods

Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger»

Anaplasma phagocytophilum, marginatum; Bartonella henselae; Borrelia burgdorferi, afzelii, garinii; Coxiella burnetii; Ehrlichia chaffeensis; Francisella tularensis; Rickettsia prowazekii, akari, rickettsii and*Yersinia pestis* are also known as arbobacteria. Diseases caused by these bacteria are basically zoonoses, i.e. diseases transmittable from animals to humans, and have been known as such for about 100 years (table 1). A part of the individual pathogens have not been described until the past few decades. Based on molecular biology analyses, *R. prowazekii, Ehrlichia* and *Anaplasma* are categorised as Rickettsiales, while *Bartonella* is categorised as alpha-2-proteobacteria, *Coxiella, Rickettsia grylli* and *F. tularensis* as gamma proteobacteria, and *Y. pestis* as enterobacteria [1].

Most arbobacteria grow predominantly intracellularly. However, *Borrelia* bacteria grow intracellularly and extracellularly, and *Yersinia* mainly extracellularly. The above described arbobacteria, when transmitted by ticks, show seasonal occurrence and a partly changed antigen repertoire in vector and mammal. *R. prowazekii* is transmitted by lice world-wide throughout the year.

The major clinical symptoms such infections have in common include fever, exanthema, headache, and lymph node swelling, partly a pronounced erythema at the site of the sting, and encephalitic disorders. Neutropenia and thrombocytopenia can occur later.

Treatment: Doxycycline is the treatment of choice against most of these bacteria, followed by chloramphenicol and cephalosporins. Quinolones are ineffective against *R. prowazekii*. The treatment of choice against *Y. pestis* and *F. tularensis* is streptomycin or gentamycin, and in addition doxycycline or ciprofloxacine.

C. burnetii has been dealt with separately [2]. Therefore, this pathogen is not included in the present review, neither are rare tropical and/or pure tropical diseases. The oriental flea (*Xenopsylla cheopsis*) is considered as the most effective transmitter for *Y. pestis*. More than 30 other flea species are known which can transmit *Y. pestis* as intermediary hosts, including Pulex irritans (human flea), which can play a role in human-to-human transmission. The human louse can also be a vector for transmission of *Y. pestis* [3].

The following section provides for each pathogen information on the general state of knowledge, characteristics of the pathogen, infectious disease, epidemiology, methods of detection and occurrence of the pathogen in the donor population. This is followed by information concerning all pathogens on epidemiology, defence situation, treatment and prevention in recipient populations as well as a summary evaluation.

1 *Anaplasma* and *Ehrlichia*

1.1 Current Knowledge about the Pathogen

Today, after separation into *Ehrlichia* and *Anaplasma*, the pathogens are subdivided as follows:

- *Anaplasma*: A phagocytophilum, platys, marginale.
- *Ehrlichia*: *E. chaffeensis,* with horse as the major host and infection in humans, as well as *Ehrlichia ewingii* and *Ehrlichia canis,* with dog as the major host, which seldom infect humans.

Up to 1987, an *Ehrlichia* infection was considered as a pure animal disease. Ehrlichiosis in dog was first described in Algeria in 1935 with the characteristic inclusions in monocytes in the Giemsa staining. The first infections in humans were reported as Sennetsu Neorickettsiosis in Japan and Malaysia. The first description of the disease after a tick bite in the USA (Arkansas) was done in 1986 [4].

In 1994, *A. phagocytophilum* was described as a distinct pathogen and the disease human granulocytotropic anaplasmosis (HGA) ascribed to it [5].

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Table 1. Vectors for arbobacteria

Bacteria	Transmission by				
	Tick(s)	Mosquito	Louse	Flea	Smear infection
Anaplasma *Ehrlichia*	*A. phagocytophila* *E. chaffeensis* *E. ewingii*				
Bartonella		*B. bacilliformis* (*Lutzomyia* as vector)	*B. quintana*	*B. henselae* (cat flea)	*B. henselae* *B. quintana* *B. bacilliformis*
Borrelia	*B. burgdorferi* *B. afzelii* *B. garinii*		*B. recurrentis*		
Coxiella	*C. burnetii*				*C. burnetii*
Francisella	*F. tularensis*	*F. tularensis*			*F. tularensis*
Rickettsia	*R. rickettsii* *R. conorii* *R. slovaca* *R. helvetica*		*R. prowazekii*	*R. typhi*	
Yersinia			*Y. pestis* (experimentally)	*Y. pestis* (*Xenopsylla cheopsis*, rat flea)	*Y. pestis* from open buboes and in case of plague pneumonia (also droplet infection)

1.1.1 Characteristics of the Pathogen

1.1.1.1 Structure

Both pathogens are small Gram-negative bacteria, growing intracellularly and forming inclusion bodies. The pathogen infects both human and animal cells and forms so-called morula cells if excessive growth occurs. Two forms of bacteria can be distinguished – a larger reticular form and a small form with a thick and dense cell wall. The cell wall does not contain any lipopolysaccharides. The surface porin proteins which are present in the wall can be coded by many genes and form the basis for antigen variability of the pathogens. Adhesins for mammalian cells are present, which contain hypervariable regions and carry a very variable portion of sugars so that re-infection with the same, immunologically different bacterium is possible.

1.1.1.2 Replication

Anaplasma and *Ehrlichia* replicate in cells of nymphs and adult ticks, mammals such as deer, cattle and horse as well as humans. In the blood, *Anaplasma* is mainly found in neutrophil granulocytes, macrophages and monocytes, seldom in lymphocytes, whereas *Ehrlichia* predominantly grows in monocytes and macrophages.

Ehrlichia shows a direct cytopathogenic effect in cell culture, to which part of the clinical manifestation can be ascribed. *Anaplasma* cannot be degraded within the neutrophil granulocytes. Apoptosis of these cells is delayed so that bacteria can be replicated over a period of several weeks.

1.1.2 Infection and Infectious Disease

After penetrating the skin, the pathogens grow and spread in white blood cells, and the infection manifests itself in organs such as the liver, spleen, lymph nodes, as well as kidney, lung and brain. Granuloma formation is typical of the disease.

Immune response is inhibited due to intracellular growth, the induced reduction of the number of CD4+, CD8+ and CD5+ cells, and the reduced function of the T lymphocytes. After approximately 30 days, the immune response against the pathogen can be determined via the formation of specific antibodies.

The mean incubation period is 1–4 weeks. The major symptoms of the infection are fever, headache, myalgia and malaise. If the lungs are involved, infiltrates can be detected in the X-ray examination. The average duration of the disease is 9 days, with a range of 1–60 days. Clinical outcome varies from mild symptoms to death. The outcome tends to be more severe at an advanced age of the patients.

1.1.3 Epidemiology

Ehrlichia is transmitted by tick. Transmission reaches its peak from May to August. *Ehrlichia* can be transmitted by the tick species of *Amblyomma americanum*, *Dermatocentor*, and *Ixodes*. *Ehrlichia* infections have mainly been found in North America and South-East Asia. *Anaplasma* is geographically distributed world-wide in regions infested with ticks. Approximately 2% of the ticks in Germany carry *A. phagocytophilum* [6]. Depending on the region, *Ixodes ricinus* ticks carry between 1 and 16% *Ehrlichia* and *Anaplasma*, the highest rates being present in ticks that live in dunes [7].

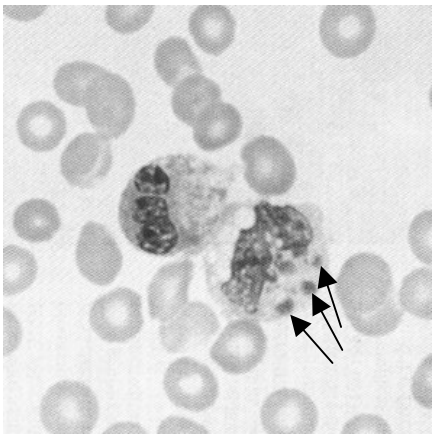

Fig. 1. *Ehrlichia*: Typical dots in mononuclear cells of peripheral blood caused by intracytoplasmic growth of *Ehrlichia* as indicated by the arrows. Shown is a detail of the figure which had been published previously in Clin Microbiol Rev 2003;16:39 [92].

1.1.4 Detection Methods

1.1.4.1 Microscopy and Blood Smear
After Giemsa staining, morulae in *Ehrlichia* can be detected as multiple, round to oval inclusion bodies, measuring 1–3 μm, in the cytoplasm of monocytoid cells of blood and bone marrow (fig. 1). The method is very insensitive, with a sensitivity of approximately 30% [8].

1.1.4.2 NAT
Polymerase chain reaction (PCR) is well suitable for clinical diagnostics. The starting material used most frequently is 16s rRNA, and the primers HE1 and HE3 are used for amplification [9]. Since only few nucleotide exchanges within 1,500 bp of 16s rRNA occur between *Ehrlichia* and *Anaplasma*, distinction between these two pathogens is barely possible using this type of PCR. Specific primers have been developed for the distinction between these species [10].

1.1.4.3 Antibody Detection
Immunofluorescence test slides are commercially available. Immunoblots have been developed to increase specificity [11]. ELISA and immunoblot have been described for *Anaplasma*. Their diagnostic validity remains open. Serological cross-reactivity between *A. marginale* and *A. phagocytophilum* in cattle is 63% [12].

1.2 Blood and Plasma Donors

1.2.1 Prevalence and Incidence in Donor Populations
Up to now, no transfusion-associated transmission of *Anaplasma* or *Ehrlichia* has been reported in Germany. Blood donors are not tested for the presence of *Anaplasma* or *Ehrlichia* bacteria, or antibodies against the latter.
In the USA, *Ehrlichia* has been transmitted via liver and pancreas transplantations [13]. In blood donors with tick bites in endemic areas in the USA, the prevalence of antibodies against *A. phagocytophilum* was 0.9% [14]. An infected tick was also found in the English Garden of Munich [15, 16].

1.2.2 Definition of Exclusion Criteria
The general exclusion criteria apply which point to an infection, such as rise in body temperature, fatigue, exhaustion, muscle pain and anaemia. If applicable, the question should be asked whether a tick bite occurred within the last 4 weeks.

1.2.3 Donor Testing

1.2.3.1 Antibody Determination
Testing for antibodies is not performed. Tests are available (c.f. '1.1.4 Detection Methods').

1.2.3.2 Detection of *Anaplasma* and *Ehrlichia*
Since cultivation requires a very high workload and is expensive but does not show enough sensitivity, PCR is the method of choice, whenever required, (cf. '1.1.4 Detection Methods').

2 *Bartonella*

2.1 Current Knowledge about the Pathogen

The bacterium is named after A.L. Barton who described the intraerythrocytary occurrence of *Bartonella quintana* in 1909. *Bartonella* is the causative agent for the bacillary angiomatosis and the so-called cat-scratch disease. Human pathogen species include *Bartonella bacilliformis, B. henselae* and *B. quintana*. A closer genetic relationship exists with *Brucella* and *Agrobacterium*.
B. bacilliformis only occurs in the Andes of South America at altitudes of between 1,000 and 3,000 m and is transmitted by ticks and midges (species: *Lutzomyia*). Clinical manifestation is known as Oroya fever or Verucca peruana.
B. quintana is transmitted by lice (*Pediculus humanus*) and is found in persons with a low standard of hygiene. The geographical distribution of *B. quintana* is world-wide.
B. henselae also occurs world-wide. Cats seem to be an animal reservoir. In the case of a bacillary angiomatosis, cats very frequently also suffer from bacteraemia. Transmission of *B. henselae* to humans is associated with contact to cats.

2.1.1 Characteristics of the Pathogen

2.1.1.1 Structure
Bartonella is a small 0.6–1.0 μm long Gram-negative rod which can also appear curved. The surface is covered with pili, but no flagellae are present. The bacterium uses the pili e.g. to adhere to endothelial cells.

2.1.1.2 Replication

Bartonella replicates inside and outside human and animal cells, and in the vector. Outside the body, *Bartonella* shows demanding growth. Within the cell, *Bartonella* destroys the cytoskeleton. Infection of endothelial cells is common in all mammals.

2.1.2 Infection and Infectious Disease

The incubation period of *B. quintana* and *B. henselae* is 3–38 days. Acute signs of illness include chills and fever. The fever persists approximately 1–3 weeks. Febrile episodes may take up to 6 weeks. Other manifestations of the disease may include headache, pain in the retrobulbar region, swollen lymph nodes, nystagmus, myalgia, arthralgia and hepatosplenomegaly. Chronic infection with relapsing fever-like episodes may frequently occur in immunocompromised patients. *Bartonella* frequently causes endocarditis. The affected patients are usually afebrile.

Epitheloid angiomatosis manifests itself locally on the skin or in the lymph node. Lesions can be few millimetres or centimetres in diameter and can be similar to Kaposi sarcoma. A typical sign of *Bartonella* is the manifestation of swollen regional lymph nodes. Encephalopathy is very rare as well as death caused by *B. henselae*. Chronic outcome of the disease over several months has only been described in immunosuppressed patients (e.g. by HIV).

Approach to Treatment: Tetracycline, rifampicine and macrolides, preferably azithromycin, are the treatments of choice. In addition, cephalosporins and quinolones may be effective.

2.1.3 Epidemiology

As described above, *B. bacilliformis* only occurs in the South American Andes. The wild animal reservoir for *B. bacilliformis* has not been identified. *B. henselae* and *B. quintana* occur world-wide. Cats are major animal hosts for *B. henselae*. The disease can be prevented by avoiding contact with vectors or cats. Relapses after antibiotic treatment may occur so that a re-occurrence of *Bartonella* infection should not automatically be considered as a new infection.

In Italy, the prevalence of *B. henselae* antibodies in exposed patients has been indicated as 6% [17]. In Swedish elite rangers who stood out due to their occurrence of unexpected cardiac death, the prevalence was 30% for *Bartonella elizabethae*, 3% for *B. henselae* and 1% for *B. quintana* [18].

2.1.4 Detection Methods

2.1.4.1 Antibodies

IgG and IgM Western Blot assays have been developed, with a specificity of 70–95% [19]. The most frequent detection test is performed using immunofluorescence with intracellularly (on Vero-cells) growing *Bartonella* as substrate. Sensitivity is approximately 90%. ELISA is commercially available. High cross-reactivity exists between antibodies against *B. henselae* and those against *B. quintana*.

2.1.4.2 Culturing in Medium

If the transport period to the laboratory is short, *Bartonella* grow in sophisticated culture media such as fresh blood agar and chocolate agar, manifesting themselves as small yellowish, smooth pleomorphic colonies. Blood culture is positive in the case of bacteraemia. *Bartonella* has been isolated from tissues such as liver, spleen, lymph nodes and skin.

Microscopically, Gram-negative rods can be seen but also round shapes.

2.1.4.3 NAT

A PCR method has been described by Handley and Regnery [20] to distinguish between pathogenic *Bartonella* species.

2.2 Blood and Plasma Donors

2.2.1 Prevalence and Incidence in Donor Populations

Bacteriaemia can develop in chronically infected asymptomatic individuals over a long period. A report is available describing the transmission of *Bartonella* by blood transfusion in cats [21]. No studies are available on the prevalence in blood donors in Germany. Transmissions have not been reported.

2.2.2 Definition of Exclusion Criteria

Typical non-specific symptoms of an infection are general exclusion criteria. Skin efflorescence pointing to angiomatosis should be another criterion for temporary donor exclusion.

3 *Borrelia*

3.1 Current Knowledge about the Pathogen

Borrelia is an intracellularly and extracellularly growing Spirochaeta-like bacterium, which occurs world-wide and is essentially transmitted by ticks in North America, Europe and Asia (*Ixodus* and *Dermatocentor* species). The association of tick bite, infection with *B. burgdorferi* and acute Lyme disease was found in 1976 [22]. In 1941, Bannwarth [23] described inflammatory polyneuritis caused by *Borrelia*. Herxheimer and Hartmann [24] described acrodermatitis chronica atrophicans in 1902, and Afzelius [25] described erythema chronicum migrans in 1909.

B. burgdorferi sensu lato is subdivided into 3 pathogenic groups: *B. burgdorferi* sensu strictu, the only strain in Northern America, and in addition, the *B. afzelii* and *B. garinii* groups in Europe. Up until recently, *Borrelia valaisiana* was classified as non-pathogenic.

3.1.1 Characteristics of the Pathogen

3.1.1.1 Structure

Borrelia carries a cytoplasmatic membrane, surrounded with periplasma containing the flagella insertion, and an outer membrane the components of which are not firmly connected with the periplasma. *Borrelia* has a diameter of 0.3–0.5 µm and a length of 8–30 µm. It carries 7–11 flagellae. The linear chromosome is approximately 950 kb in size. Nine circular and 12 linear plasmids contribute to the genome. *Borrelia* contains many lipoproteins such as Osp A to F (outer surface protein) [26]. Various proteins are expressed in different ways during an infection, e.g. VIsE (variable major protein-like sequences expressed) [27] and Salp15 [28].

3.1.1.2 Replication

Borrelia grows both intracellularly and extracellularly on mammalian cell cultures under microaerophilic and anaerobic conditions. *Borrelia* can also be cultured as biopsy material in Barbour-Stoenner-Kelly-II culture medium. Up to now, it has not been possible to achieve replication in solid culture media. The period of generation is 7–20 h at 30–33 °C [29]. Each human cell can be infected, including synovial cells.

3.1.2 Infection and Infectious Disease

Clinical pathogenicity partly derives from the expression of different proteins. Thus, Osp C is barely present in the tick but is highly expressed in the mammal. The same applies to the VIsE. Since these antigens constantly change, the immune response is compromised time and again. Infection occurs by tick bite. 3–32 days later, *Borrelia* replicates in local tissue, usually the skin. *Borrelia* spreads in the body within a few days or weeks, which can lead to bacteriaemia and also infection of the central nervous system. Further preferred replication sites include the myocardium, liver, muscle, retina and spleen.

Osp C, DbpA and DbpB (decorin binding protein), fibronectin binding protein (BBK32 – 47 kDa protein) and VIsE are responsible for the induction of inflammation mediators, depending on the strain.

The disease is subdivided into three stages:

Stage 1 Local Infection: In >50% of the infected individuals, erythema chronicum migrans develops, even though a tick bite is not remembered, since nymphs fall off after feeding on blood. The centre of the erythema may cause a burning or itching sensation and does not necessarily show the characteristic reddening.

Stage 2 Disseminated Infection: After a few days or weeks after the tick bite, multiple lesions develop on the skin as a sign of the haematogenous seed, together with fever, fatigue, exhaustion, and regional lymph node swelling. Meningeal irritation is possible and also hepatitis, splenomegaly, cardiomyopathy, osteomyelitis and panophthalmitis.

Stage 3 Persistent Infection: Months or years after the tick bite, intermittent swelling of the joints, arthritis, axonal neuropathy, encephalomyelitis, with ataxia and cognitive disorders occur, or years after infection, acrodermatitis atrophicans with red painful sclerosing or atrophic induration of the skin. *Borrelia* can be detected in the tissue affected even after long persistence.

Borrelia can be transmitted to the foetus during pregnancy [30].

3.1.3 Epidemiology

In the northern hemisphere, *Borrelia* is wide-spread in North Africa, Europe, including Scandinavia and Russia, China and Japan, and in many other countries. Depending on the endemic area and lifestyle, 1–2% of the population can be infected. Prevalence is higher among forest workers. In the Bavarian Forest e.g. it is 30% [31], and in Italy 7% compared with the general population in the Toscana of 3% [32]. The population in the province around Madrid in Spain also showed an antibody prevalence of 3% [33].

In Southwest and Central Asia, further *Borrelia* strains, e.g. *Borrelia recurrentis*, are transmitted and cause recurrent fever, usually by ticks such as *Ornithodorus* and the lice, *P. humanus*.

In Germany, *Borrelia* occurs in all regions in which ticks live. Up to 30% of the ticks can be infected with the 3 species *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. On average, 11% of the ticks are infected in central and southern Germany. Older ticks have a higher infection rate. The predominant of the three species is *B. garinii* with 60%, followed by *B. burgdorferi* with 32%, and *B. afzelii* with 18% [6]. *B. valaisiana* can occur in 6% of the ticks. Double and few triple infections also occur [6]. In the Netherlands, the infection rate of ticks is between 0.8 and 11.5% [7].

People in all age groups can be infected. Depending on the geographical region, seasonal tick activity in Germany lasts from May to October or from March to November.

3.1.4 Detection Methods

3.1.4.1 Antibodies

The screening test generally used is ELISA, which contains bacterial lysate, recombinant proteins, or a combination of the latter, depending on the manufacturer. IgG and IgM can be detected; IgM can persist over a period of months up to several years. False-positive reactions are not unusual. Therefore, ELISA reactivity is confirmed via immunoblot. Several interpretation criteria have been determined for the immunoblot [34].

3.1.4.2 Indirect Immunofluorescence Assay

The indirect immunofluorescence assay serves as specificity control. Antibodies from patients are absorbed with *Treponema phagedenis*, and then incubated on slides with *Borrelia* cultivated in medium. Antibody titres must be quantitatively evaluated. Experience is required for correct interpretation.

3.1.4.3 Immunoblot
Currently used immunoblot strips are supplemented with recombinant VIsE [27]. The main indication for the use of immunoblots is the clarification of false-positive ELISA results and the serological clarification of neurologic borreliosis.

3.1.4.4 Culturing in Medium
Culturing may be successful in Barbour-Stoenner-Kelly-II medium [29]. This is very time-consuming and requires a high workload on the part of the staff. It is therefore not routinely used.

3.1.4.5 Microscopy
Microscopic analysis shows *Borrelia* as weakly stainable, Gram-negative motile rods. In the blood smear, *B. recurrentis* can normally only be seen during the acute infectious phase.

3.1.4.6 NAT
PCR or derived methods are the methods of choice for the detection of *Borrelia*. PCR can be carried out in all tissues, partly after enriching the 16s DNA.
Sensitivity is between 80 and 95% [35], depending on the primer selection from the Osp A. There is a range of sensitivity between 50–75% dependent on the species, i.e. when *B. burgdorferi* primers are used for the amplification of *B. garinii* or *B. afzelii* Osp A-gene [36].

3.1.4.7 Animal Experiments
Borrelia grows in mice, particularly in the gerbil, and also in rats and hamsters.

3.2 Blood and Plasma Donors

3.2.1 Prevalence and Incidence in Donor Populations
Seroprevalence of blood donors in Germany and Switzerland does not differ from that of the local population. It was 2.7% in Hamburg and Würzburg and 33% in Solothurn [37–39]. Nine blood recipients had no antibodies against *Borrelia* [38] after seroconversion of the donor during follow-up, pointing to low transmission effectiveness. No transmission of *Borrelia* by transfusion has so far been reported in Germany.
In case of bacteriaemia, *Borrelia* can certainly survive at 4 °C during the storage period of the red blood cells, as shown in studies performed by Johnson et al. [40] and by Nadelman et al. [41]. A study in an endemic region such as Connecticut on the risk of *Borrelia* transmission by blood transfusion showed that practically no transmission takes place [42].

3.2.2 Definition of Exclusion Criteria
After *Borrelia* infection by tick bite followed either by local or the usual general signs of inflammation, Lyme disease with its clinical manifestations of fever, fatigue, and exhaustion will be detected by the usual exclusion criteria.
If required, the donor can be asked whether a tick bite occurred within the past 6 weeks, however, this is not a reliable exclusion criterion since, as mentioned above, nymphs fall off after the bite, leaving it unnoticed.

3.2.3 Donor Testing and Significance
To date, blood donors have not been tested routinely for the presence of *Borrelia* antibodies. The current epidemiological situation still does not justify general donor screening for *Borrelia* [38].

3.2.3.1 Antibody Determination
Borrelia antibody determination using ELISA is very sensitive if an incubation period of approximately 4–6 weeks has elapsed. Immunoblot is carried out to exclude false-positive reactions (cf. 3.1.4 'Detection Methods').

3.2.3.2 Direct Detection for *Borrelia*
If an infection is suspected, detection is best carried out with plasma after enrichment via centrifugation or by means of fine-needle biopsy from the inflamed skin around the tick's stinging site. General donor testing for *Borrelia*, e.g. using PCR, is not required.

3.2.4 Donor Interviews
Donors are partly interviewed on a possible exposure after tick bite. This is of little informational value during the winter months. Other signs of bacterial infection, e.g. fever, are recorded based on the usual exclusion criteria.

3.2.5 Donor Information and Counselling
Comprehensive advice is necessary only if the tick bite has been remembered as having occurred within the most recent 1–2 months. In addition to information on *Borrelia*, this advice should also include other relevant arbobacteria and also arboviruses such as tick-borne meningo-encephalitis virus [91].

4 *Francisella tularensis*

4.1 Current Knowledge about the Pathogen

The genus *Francisella* is part of the sub-class of gammaproteobacteria and the order of Thiotrichalis. It forms a family in its own right: the Francisellaceae which is closely related to the family of the Piscirickettsiaceae, and also represents the next relative of the order of Legionellalis (*Coxiella, Rickettsiella*). The bacterium was isolated by McCoy and Chapin [43] from a dead gopher in Tulare, CA, in 1910. 10 years later, Frances [44] described the disease more accurately as deer-fly fever, and also described the human infection [44]. *Francisella* occurs endemically in the entire northern hemisphere, especially in animals living near or on the ground such as rodents and more than 100 other mammals. The natural reservoir has

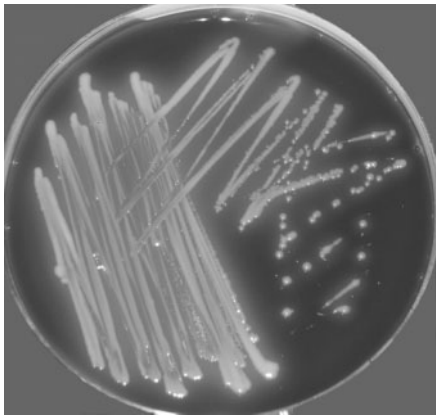

Fig. 2. Cystein-heart-blood agar plate with colonies of *F. tularensis* (PD R. Grunow, Robert Koch-Institute, Berlin, Germany).

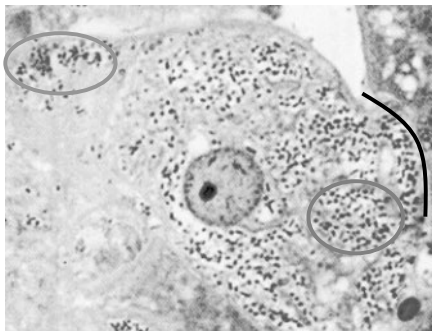

Fig. 3. *Francisella:* Intracellularly accumulated Gram-negative small red shaped bacteria shown within the ovals and restriction to the cytoplasm of one cell as indicated by the black line. Shown is the section of a figure published in ASM News 2003;11:560 [49] previously.

not yet been identified. It is, however, assumed that the pathogen persists in the environment under certain circumstances and in certain animal organisms over a period of months or years.

In addition to transmission by contact with infected animals, laboratory infections also occurred [45]. *Francisella* is transmitted through arthropods as vector, including ticks and midges, as well as by direct contact with infected animals and contaminated environmental materials [46]. Transmission from human to human is unlikely.

There are two species within the *Francisella* genus: *Francisella philomiragia* and *F. tularensis*. The latter is subdivided into 4 sub-species of which *F. tularensis* ssp. tularensis (occurrence in North America) and ssp. holarctica (occurrence in Europe and the entire northern hemisphere) are predominantly human pathogenic [47]. These pathological pictures are related to the entry site of the pathogen and are often characterised by lymph node swelling and skin ulcers. *F. philomiragia* was found in water and soil samples in Texas [48].

F. tularensis ssp. tularensis is considered as hazardous agent potentially usable as biological weapon, and requires culturing under level 3 biosafety laboratory conditions.

4.1.1 Characteristics of the Pathogen

4.1.1.1 Structure

Francisella is a relatively small, faintly stainable, Gram-negative, coccoid, aerobically growing, greyish-white, colony-forming, catalase-positive bacterium. It is immobile, does not form spores and has an electron-transparent lipid-rich capsule which contributes to the virulence of the pathogen.

4.1.1.2 Replication

Francisella grows in the laboratory in sophisticated culture media with blood and a high cystein content, preferably heart-cystein-blood agar, but also chocolate agar or Thayer-Martin agar, which is suitable for gonococci. The culture grows at 37 °C into greyish-white shiny colonies, approximately 3–5 mm in size, in a humid atmosphere with 5–10% CO_2 within 2–5 days. (fig. 2).

10–50 organisms form 1 HID (human infectious dose) after inoculation or inhalation. Approximately 10^8 organisms are required for infection via food. After ingestion, the bacteria replicate intracellularly in lymphoid tissues. Cells of the reticulohistocytic system represent the primary target cells, but hepatocytes are also infected (fig. 3) [49]. Immune response is primarily T lymphocyte-mediated. Antibodies indicate that exposure has taken place. Papule, ulcer and granuloma formation, which may necrotise at a late stage, are characteristic for the disease.

In water, *Francisella* can replicate in amoebae [50]. Infected amoebae are not killed, however, *Francisella* loses some of its virulence in the mouse test by passing into the amoeba.

The bacteria retain their stability under cool and humid conditions for several weeks to months. The pathogen is sensitive to commercial disinfectants and heat treatment. Typically, the bacteria can be killed when treated at temperatures as low as 60 °C for 1 h.

4.1.2 Infection and Infectious Disease

Clinical manifestation essentially depends on the number of inoculated bacteria, their virulence and the host's immune response. The spectrum of disease varies from asymptomatic infection over severe sepsis to death. The incubation period is 3–5 days on average, and ranges from 1–21 days. The infection can be accompanied by sudden occurrence of fever, chills, headache, fatigue, cough, myalgia, breathing trouble and diarrhoea. The fever rises above 39 °C and persists for a few days. Activation of the MgIA-regulated genes during growth in the macrophages plays an important part for virulence and pathogenicity [51]. As a rule, the disease can be well treated with an appropriate antibiotic. Depending on the type of pathogen, the lethality rate is as high as 30% or higher in untreated cases [52].

The ulceroglandular form with the typical swelling of the regional lymph nodes develops after inoculation of the pathogen via arthropods or direct contact with contaminated animals, carcass, excrements or other environmental materials. All signs of inflammation accompany the main ulcer, the edge of which hardens. Necrosis in the lymph node may suppurate and

break through towards the interior or the exterior. Super-infections with other bacteria are not unusual.
The oculoglandular form occurs relatively seldom and is usually caused by smear infection. In rare cases, contaminated dusts or aerosols may be the cause.
Oropharyngeal tularaemia manifests itself after oral ingestion of *Francisella*-contaminated food or water. In addition to general symptoms, an almost exclusively one-sided cervical lymph node swelling is characteristic of the disease.
Respiratory tularaemia occurs after inhalation of contaminated dusts and aerosols, usually during farming activities and presents the most severe clinical outcome of tularaemia. In addition to general symptoms, it can manifest itself as a pneumonia-like disease with cough, chest pain and increased respiratory rate. *F. tularensis* can replicate directly in the alveolar epithelial cells after inhalation [53]. Secondary pneumonia after haematogenic spread of the pathogen is also possible. If the pneumonia persists for longer periods, granuloma formation in the X-ray picture can resemble that of tuberculosis.
The term 'typhoid tularaemia' was applied in the past if no typical external signs of tularaemia were visible. Today, this term should only be used in certain circumstances if the route of infection cannot be detected.
Skin erythema occurs in a third of the *Francisella*-infected population 2–3 weeks following infection. This is a manifestation of the bacterial spread and incipient immune response. Absceding and liquefying lymph nodes occur even if the acute signs of illness have subsided.

4.1.3 Epidemiology

F. tularensis has its geographical distribution in the entire northern hemisphere. Persons with a particularly high risk include hunters, farmers, foresters and other groups exposed to outdoor activities for professional or leisure reasons. Among animals, rodents are the preferred reservoir for *Francisella*, but the pathogen also occurs in other mammals and birds. Besides direct contact, arthropods also play an essential part as vectors in the transmission to humans. A prevalence of 0.1–2% has been described for European ticks [54]. Thus, 2% of the ticks in endemic areas are infected with *Francisella* in the Czech Republic and in Austria [55]. Transmission occurs by tick bite of the *Ixodes, Dermatocentor, Amblyomma* species as well as midges of the *Aedes, Culex,* and *Anopheles* species [46]. A third of the 27 hunters and herders in a hare hunt developed *F. tularensis* infection, partly with acute manifestations, in the 'Landkreis' (rural district) of Darmstadt-Dieburg [56] in Hesse, Germany, in 2005 [56].
The occurrence of *Francisella* in freshwater amoebae and the possibility of spreading in water are addressed in Section 4.1.1.2.

4.1.4 Detection Methods

4.1.4.1 Antibodies

Approximately 2 weeks after the occurrence of the clinical symptoms, IgG, IgA, and IgM antibodies can be detected simultaneously. The antibodies thus formed persist for more than 10 years. Available rapid tests include tube or plate agglutination tests the sensitivity of which does not exceed 100% [57]. ELISA and Western Blot assays are used as screening and confirmation tests. These tests are highly sensitive and specific for the holarctica and tularensis subspecies. However, they are currently not yet commercially available and must be adapted to European or North American *Francisella* strains, depending on the requirements [58, 59].

4.1.4.2 Culturing in Medium

F. tularensis does not grow in standard bouillon such as Luria Bertani (LB). The bacterium grows into colonies on blood culture medium enriched with cystein within approximately 48 h at 37 °C under aerobic conditions with 5–10% CO_2. For clinical samples, culturing is best carried out by using smears of skin ulcers or blood. Targeted culturing requires laboratories with biosafety level 3 (in Germany, however, the holarctica subspecies is classified as a pathogen of biohazard level 2). Accompanying flora suppresses the growth of *Francisella*. Suitable mixtures of antibiotics for the shielding of cultures, especially those from environmental samples, are currently being developed. Environmental samples should first be concentrated using 0.45 or 0.22 µm filters, followed by cultivation of the filtered material.

4.1.4.3 Animal Experiment

Another way of culturing is the inoculation of the mouse with material to be tested. *F. tularensis* will grow in the mouse within one week.

4.1.4.4 NAT

Several commercial PCR tests for the detection of *F. tularensis* identify the genomic region of *Francisella* outer membrane protein fopA [60] or the 17-kDa lipoprotein. Differentiation of the subspecies is in most cases successfully carried out using RD1-PCR [62]. PCR is more sensitive than culturing. A TaqMan method for the detection of the bacterium in mammals and vectors has been described [63], and so have a number of other molecular methods for typing *Francisella*.

4.2 Blood and Plasma Donors

4.2.1 Prevalence and Incidence in Donor Populations

In Germany, no transmission of *F. tularensis* via blood has been described to date. A report on the transmission of *F. tularensis* via a kidney transplant is available from Saint Louis, MO, USA. In this report, the infection in the recipient was suc-

cessfully treated with antibiotics following the diagnosis in the donor [64].

4.2.2 Definition of Exclusion Criteria

The general exclusion criteria for donors apply to donors with signs of infection. The main cause of contracting *Francisella* infection in Germany is an injury during gutting of infected hares or the consumption of contaminated meat from hare [56].

4.2.3 Donor Testing and Significance

Because of the epidemiological situation with very low infection numbers and without any known transmission of *F. tularensis* via blood in Germany, testing is currently not required.

5 *Rickettsia*

5.1 Current Knowledge about the Pathogen

Rickettsia is a small Gram-negative obligate intracellular parasite found in mammals, belonging to the alpha proteobacteria species. From the family of *Rickettsia*, the species *Ehrlichia*, *Anaplasma*, and *Orienta* (*Orienta tsutsugamushi*) have been separated based on genetic analyses. The various different *Rickettsia* types are partly categorised into the spotted fever group (SFG – *Rickettsia africa, Rickettsia conori, Rickettsia sibirica, Rickettsia slovaca, R. rickettsii, Rickettsia honei* and *Rickettsia japonica*) and the typhoid group (TG – *Rickettsia typhi* and *R. prowazekii*). This classification has not yet been internationally recognised (table 2).

5.1.1 Characteristics of the Pathogen

5.1.1.1 Structure

Rickettsia is a genus of Gram-negative bacteria that presents as rods of 0.4 to approximately 1.5 µm in length. *Rickettsia* is faintly stainable and not easily visible if Gram staining is applied so that the preferred method is acridine orange staining. The genome is very small, with 1.1–1.6 Mbp. The cell wall contains lipopolysaccharides, peptidoglycans and the outer membrane protein P (Omp P), a small surface protein with approximately 135 kDa, and a 17 kDa lipoprotein [65]. The proteins Omp A and Omp B are only intermittently expressed on the surface during maturation of the bacteria. The lipopolysaccharides of the different *Rickettsia* lead to immunological cross-reaction. *Rickettsia* carry a carbohydrate layer on their surface. They are freely motile within the mammalian cell. In the vector, *Rickettsia* bacteria are transmitted transovarially so that, if they are present in large quantities and replicate strongly, horizontal transmission to the infected mammal is also possible [66].

Table 2. *Rickettsia* with significance for Europe

Rickettsia	Vector	Disease
R. akari	midge	rickettsial pox
R. conorii	tick	boutonneuse fever, tick bite fever
R. helvetica	tick	spotted fever with slowly healing papules
R. slovaca	tick	spotted fever with slowly healing papules
R. felis	cat flea	cat flea spotted fever (except for humans)
R. prowazekii	louse	epidemic spotted fever, Brill-Zinsser disease – which occurs years after infection, cross-reaction with Proteus OX19 (Weil Felix reaction). The louse dies of the R. infection, louse faeces are infectious.
R. sibirica	louse, tick	spotted fever with slowly healing papules
R. typhi	flea	murine spotted fever; 'Fleckfieber'
R. rickettsii	tick	Rocky Mountains spotted Fieber (only North America – patient's travel history)

5.1.1.2 Replication

Rickettsia replicates in cells of the vector and in great numbers of cells of the infected mammal. *Rickettsia* can be cultured and replicated on Vero, HEL and L929 cells.

5.1.2 Infection and Infectious Disease

Rickettsia bacteria are spread via the lymph and blood pathways following inoculation. They adhere to the surface of endothelial and other cells via the proteins Omp A and Omp B. They are phagocytised, escape lysis in the phagosome and move into the cell core. There, they replicate by longitudinal division, are moved to cell surface along the actin skeleton and then released. Cell-to-cell transmission, e.g. in the skin and the lungs, is possible. The infected cell dies after a few days so that bleeding may result after endothelial cell infection. Whether the disease is overcome depends on rapid availability of cytotoxic CD8+ T lymphocytes from the host.

In addition to the skin and the vascular system, manifestation sites of a *Rickettsia* infection include interstitial pneumonia, interstitial myocarditis and perivascular haemorrhage in the brain, gastrointestinal duct and kidney.

The incubation period is 2–14 days, on average 7 days. The body temperature rises above 39 °C.

In case of an *R. conori* infection, clinical outcome is usually mild. The course of the disease of this bacterium is similar to spotted fever. The bacterium occurs in South-East Europe, Africa, the Middle East up to Central Asia. The incubation period is 5–7 days. Clinical signs that stand out the most include swollen lymph nodes with a tendency to superficial haemorrhage at the inoculation site. In the body, the bacterium is usually spread via the blood pathways. The endothelial cells are massively infected. Deaths may occur.

The incubation period of *R. akari* is 10–17 days. The bacterium occurs in North America and Central Europe. In case of *R. akari*, one singular papule per inoculation is formed per bite

from which a vesicle develops which droops centrally, bleeds inward and forms a scab. Regional lymph nodes are swollen. The disease is described as *Rickettsia* pox.
R. prowazekii is the pathogen of the endemic spotted fever (typhus), which has a world-wide distribution. Symptoms include spotted rash and fever which persists 8–10 days and can continue at a level of up to 40 °C. The incubation period is 7–14 days. Initially severe headache and limb pain and the skin exanthema as from day 4–7 are typical symptoms. Depending on the nutritional situation of the infected individual, the death rate can be high (Napoleon army: more than 100,000 deaths). The patient often does not recover entirely. Relapses will then occur frequently, the so-called Brill-Zinsser disease (relapsing disease first described by Nathan Brill in New York in 1898 [67]). The epidemiological connection was established by Hans Zinsser in 1933 [68].

5.1.3 Epidemiology
R. akari and *R. prowazekii* occur world-wide. Transmission depends on the spreading of the vectors and the hygienic conditions. The concerned vector regions remain infectious for decades due to transovarial transmission since agricultural animals are also affected [69]. Wild-animal populations are only partly infected.

5.1.4 Detection Methods

5.1.4.1 Culturing in Media
If there is a history of the exposure and clinical symptoms, *Rickettsia* detection should be performed as early as possible during the infection phase by drawing heparin blood and transferring it onto culture cells. Approximately 0.5 ml heparin blood or extracts of tissue samples are transferred to Vero, L929, HEL, or MRC5 cells before starting treatment of the patient with doxycycline. First growth of the bacteria can be seen after 2–3 days. Detection of *Rickettsia* is carried out by immunofluorescence or immune peroxidase staining. Culturing *Rickettsia* requires a biosafety level 3 laboratory.

5.1.4.2 Antibodies
Indirect immunofluorescence with commercially available *Rickettsia*-infected cells is still the standard. IgM and IgG detection are carried out separately. In addition, antibodies can be detected by latex agglutination (*R. rickettsii*) and ELISA: ELISA assays for the various different *Rickettsia* types, including *Orienta tsutsugamushi*, are commercially available.
When evaluating the results, cross-reactions such as Weil-Felix reaction of *Proteus vulgaris* and *Proteus mirabilis* must also be taken into account. For the detection of an acute infection, it is the increase in titres that is of importance.

5.1.4.3 NAT
PCR is the most reliable method of *Rickettsia* detection. Reliable results are obtained if the gene of the 17 kDa lipoprotein is amplified [70]. The results can be obtained in special laboratories within 6 h [71].

5.2 Blood and Plasma Donors

5.2.1 Prevalence and Incidence in Donor Populations
Routine testing of donors for antibodies or the bacterium is currently not indicated due to the current epidemiological situation. Antibodies against *Rickettsia* have been identified in blood donors in South France (prevalence 5–20% [72]) and Malaysia (prevalence 15% [73]). The *Rickettsia* antibody prevalence depends on the region. Thus, 8% was described for *R. conorii* and 2% for *R. typhi* in North Greece [74], 7% [75] for *R. typhi* in Central Spain and 74% [76] for *R. conorii* in West Spain.
Since *Rickettsia* replicates in endothelial cells, it can be transmitted by blood transfusion in the acute, pre-clinical and relapsing phases. A case of *R. rickettsiae* transmission by blood transfusion was described in 1978, in which the recipient survived after treatment with antibiotics; the donor, however, did not [77].

5.2.2 Definition of Exclusion Criteria
History of tick bites, louse bites, louse infestation, and visits to endemic areas are valid exclusion criteria. The same applies to typical healing papules and scratch marks on the skin.

5.2.3 Donor Testing and Significance
In Germany, donors are not tested for *Rickettsia* antibodies or *Rickettsia* nucleic acid, and this is not required based on the current epidemiological situation.

5.2.4 Donor Interviews
In case of suspected infection, targeted questions on louse infestation and tick bite can contribute to the prevention of *Rickettsia* transmission by blood donation.

5.2.5 Donor Information and Counselling
Advice is indicated, if the donor's history requires this. This advice can also be given by physicians in an external centre with specialised knowledge.

6 *Yersinia pestis*

6.1 Current Knowledge about the Pathogen

Yersinia belongs to the family of Enterobacteriaceae. As psychrophilic bacterium, *Yersinia* is capable of replicating at 4 °C. This ability plays an important part in transfusion medicine. Especially in the case of *Yersinia enterocolitica*, it can lead to a replication if stored in blood reserves [78]. *Yersinia* resides in the soil as well as in animals such as rodents, pigs and birds

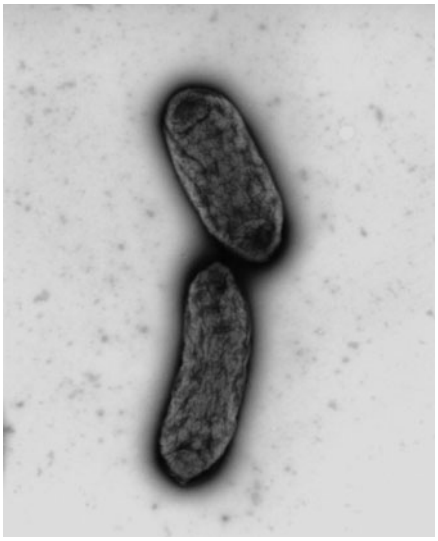

Fig. 4. *Y. pestis*. Photo taken using transmission electron microscopy. The length of the bacterium is approximately 1–2 μm.

[79]. The plague, caused by *Y. pestis*, occurred in three major pandemics in history: Justinian Plague (531–580 AD), the plague of the 14th century, also known as 'The Black Death', and the Hong Kong Plague, which killed more than 100 million people [80].

6.1.1 Characteristics of the Pathogen

6.1.1.1 Structure

Y. pestis is a Gram-negative rod-shaped bacterium with a bipolar staining (giving it a safety-pin appearance, fig. 4). There are three distinct biovars of *Yersinia*: antiqua, mediaevalis, and orientalis. The virulence of *Y. pestis* depends on the presence of different plasmids. The 70 kb virulence plasmid encodes for the expression of the type-III secretion system, which lyses macrophages by contact-dependent injection of effector proteins into these macrophages, and for the V antigen. The 110 kb plasmid encodes for the factor of the anti-phagocytic fraction (Fra 1), and the 9.5 kb plasmid encodes for the temperature-dependent protease (plasminogen activator protein – Pla protease) [80, 81].

6.1.1.2 Replication

Y. pestis grows faster at 28 °C and should be cultivated at 37 °C for at least 48 h. Virulent isolates grow at 37 °C as small colonies compared to avirulent ones. The expression of the genes is controlled on the plasmids depending on the growth temperature, thus influencing the virulence.

6.1.1.3 Extent to which the Bacterium Can Be Inactivated; Stability under Environmental Conditions

For general information, please refer to section 1.1.3. Similarly to other arbobacteria, e.g. *C. burnetii* and *Francisella*, *Y. pestis* is very resistant in the environment. The bacterium grows at temperatures as from 0 °C, and also replicates in coagulated blood. However, the pathogen is sensitive to sun light and can easily be killed by high temperatures and disinfectant.

6.1.2 Infection and Infectious Disease

Pathogenicity is essentially influenced by the structure of the plasmid. This explains why *Y. pestis* strains with different degrees of pathogenicity occur in geographically restricted areas. The immune response in humans is based on antibodies and the rapid formation of cytotoxic T lymphocytes directed against virulence factors encoded by the 70 kb plasmid [82], and directed against Caf1 encoded by the 110 kb plasmid, a protein which acts as a chaperon [83].

6.1.2.1 Rat Flea

In the presence of the 9.5 kb plasmid, *Y. pestis* causes the sucked blood in the flea to coagulate, and *Yersinia* replicates in the coagulum. This leads to an inhibition of the intestinal motility so that the blood is regurgitated at the next sucking action, and thousands of *Yersinia* are injected into the skin of the rat or human.

6.1.2.2 Human – Bubonic Plague

This type of plague is the most common form of manifestation. Fever (38.5–40 °C) and chills suddenly occur within an incubation period of 2–7 days. Painfully swollen lymph nodes develop within few hours, which form part of the regional drainage area at the inoculation site of the rat flea. The size can be up to 10 cm. Affected limbs – the bite usually occurs at the extremities – are spared. Suppurating ulceration of the lymph node can occur. Papules and pustules can occur adjacent to the lymph nodes. There are signs of bacterial metastases. Bacteriaemia is detectable in 80% of the bubonic plague.

6.1.2.3 Septic Plague

If the bacteria cannot be retained in the lymph node, sepsis occurs. The patient rapidly becomes moribund. Primary sepsis without the lymph node swelling phase or without pneumonia might occur. If not treated, sepsis rapidly leads to death the preliminary phase of which is intravascular coagulation leading to peripheral suboptimal blood circulation making the skin appear deep purple, giving this disease its name 'Black Death'.

6.1.2.4 Pneumonic Plague

The lung is affected by inhalation of the pathogen or haematogenic dissemination of *Y. pestis*. Symptoms include tachypnoea, dyspnoea, cough and bloody sputum. The sputum is purulent. *Y. pestis* can be transmitted directly to individuals in the vicinity by its great number of bacteria in the secretion or sputum via smear infection or aerosol. Transmission from human to human, however, occurs very seldom. If left untreated, pneumonic plague leads to death.

6.1.2.5 Treatment of *Y. pestis*

Streptomycin or gentamycin is the treatment of choice. In addition, doxycycline and ciprofloxacine are also effective. Chloramphenicol is effective for the treatment of plague meningitis. Resistant *Y. pestis* strains are very rare in endemic regions.

6.1.3 Epidemiology

Plague is a typical zoonosis, transmitted by vector bite. Its enzootic occurrence currently includes many natural endemic areas in many countries in Africa, the two Americas and Asia. Outbreaks occur annually with 100–1,000 affected individuals. Up to now, endemic areas for *Y. pestis* can be found in the south of Africa including Madagascar, Southern Russia to India, Mongolia, China, Indonesia, the west of North America, and the centre of South America.

Essentially, the hosts are rats (*Rattus rattus, Rattus norwegicus*), and the essential carrier is the rat flea (*Xenopsylla cheopis*). Other animal hosts include the gopher, the rabbit, the field mouse, and more seldom the dromedary. Other epidemiologically relevant vectors beside the flea are not known.

6.1.4 Detection Methods

In case of suspected *Y. pestis* infection, bacteriologic diagnostics must be performed in a biosafety level 3 laboratory.

6.1.4.1 Antibodies

Antibodies are detectable 1–2 weeks after occurrence of the symptoms. In approximately 5% of all infected individuals, no antibodies are detectable. ELISA assays have been developed for IgM and IgG.

6.1.4.2 Culturing in Media

Y. pestis grows in most culture media, as mentioned above, better at 28 °C than at 37 °C. Suitable culture media are McConkey blood and modified CIN (Cefsulodin Irgasan Novobiocin) agar in aerobic conditions. The small translucent colonies are detectable after 24–48 h depending on the temperature. Differentiation is carried out via biochemically detected enzyme performance and motility of *Y. pestis*, as well as via molecular biology and immunology methods.

6.1.4.3 NAT

Several methods have been published on the detection by means of PCR; above all, under the aspect that *Y. pestis* can be used as a biological weapon. An advantage of PCR compared with the tedious biochemical characterisation is that detection is possible directly from the colony material or even the sample, and that the presence of *Y. pestis* can be safely confirmed within approximately 2–3 h. Several real-time PCR assays have been described: Chase et al. [84] has used the chromosomal marker yp48 for the distinction of *Y. pestis* from other *Yersinia* types. Other tests are based on the detection of *Y. pestis*-specific plasmids [85, 86].

According to Section 6 of the German Infektionsschutzgesetz (Infection Protection Act), the detection of *Y. pestis*, clinically suspected *Y. pestis* infection, and the disease and death due to the pathogen are notifiable.

6.2 Blood and Plasma Donors

6.2.1 Prevalence and Incidence in Donor Populations

Since *Y. pestis* only occurs in endemic areas and outside Germany, no figures have been collected for Germany. Patients infected with *Y. pestis* show signs of acute inflammation and are therefore generally excluded from blood donation.

6.2.2 Definition of Exclusion Criteria

The general criteria for donors with signs of infection apply. Lymph node swelling and signs of fever are initial symptoms of the infection. The deferral for donors returning from tropical areas only applies to part of the individuals returning from endemic areas. Interviews on the donor history may help clarify an indication to an infection.

6.2.3 Donor Testing and Significance

Based on the epidemiologic situation, no infections due to blood donation have become known in the past few decades. Thus, testing for *Y. pestis* is currently not required.

7 Facts about all Arbobacteria

Arbobacteria can retain their ability to replicate in the soil or in water in fresh water amoebae for months, thus showing great resistance to the environment. Analogous with other non-spore formers, heating to 65–70 °C for 10 min and chemical treatment with 1% phenol, 5% hydrogen peroxide, 5% chloroform, 0.5% hypochlorite, and 5% formaldehyde for 10–30 min are considered as effective for inactivation. Disinfecting with 70% ethanol for 1 min also inactivates arbobacteria.

7.1 Recipients

7.1.1 Prevalence and Incidence of Blood-Associated Infections and Infectious Diseases in Recipient Populations

Few data are available on the prevalence and incidence in recipients in Germany. Regionally different immunity, above all, exists for infections with *Borrelia* which depends on the tick occurrence, the infection of ticks with *Borrelia*, and the leisure time behaviour and profession of the recipient. For *Anaplasma, Ehrlichia, Bartonella, Francisella* and *Rickettsia*, antibody prevalences are in the low percentile. Exposure to *Y. pestis* is not assumed for persons living in Germany.

The following antibody prevalences are assumed according to a study by Raoult [1] in France:

- *Bartonella:* 0.1–0.001%,
- *Borrelia:* 1–0.01%,
- *Francisella:* 0.1–0.001%,
- *R. prowazekii:* up to 35% infection rate in homeless individuals,

– R. typhi: 0.001%,
– Ehrlichia: 0.01% in the USA [10].

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

The defence situation depends on the general state of the infected individual, his/her existing immunity, immunoreactivity, age and exogenous factors. The essential factor causing chronic outcome of the infection by arbobacteria results from the primarily intracellular growth of arbobacteria and continued change of the antigenic surface profile. Infectiousness of blood cannot be removed from blood entirely by leukocyte depletion since the bacteria are also present extracellularly.

Small amounts of free bacteria are removed by unspecific defence mechanisms, such as complement lysis, opsonisation and lipopolysaccharide-directed phagocytosis, as well as other mechanisms of the congenital immune defences. Since the defence mechanisms decrease with age, in over 60-year-olds chronic outcome and/or relapse of the infection must be expected after transmission of the infection (cf. Brill-Zinsser disease under *R. prowazekii*). Possible complications such as abscess formation, endocarditis, myocarditis, encephalitis and immunoreactive arthritis also occur more frequently in older individuals.

After transfusion of a bacterially contaminated blood reserve, first antibodies are detectable within 2–3 weeks as measurable immune response and the pathogen can be detected via NAT, at least in specialised laboratories, within 2–3 weeks at the earliest (cf. 6.1.4.3). Fulminant and rapidly deadly outcomes have been described for *Bartonella, Francisella* and *Rickettsia* infections in case of immune deficiency, especially caused by HIV [1]. For patients without immune deficiency diseases, primary major symptoms of arbobacteria transmission include high, partly relapsing fever, particularly induced by the released lipopolysaccharide (endotoxin). Fever does not necessarily occur in the case of an immune deficiency, nor does the typical lymph node swelling [87].

The further outcome for the infected receiver is basically determined by rapidly induced antibiotic treatment, as shown e.g. for *R. rickettsii* transmission by blood transfusion [77].

7.1.2.1 Vaccination

A vaccine has been developed only against *B. burgdorferi*, and has been used in approximately 450,000 individuals in the USA [88]. This vaccine has been withdrawn from the market because of the occurrence of unclarified arthritis which has been in questionable causal relationship with the vaccination. No vaccine has been available so far for humans against infection with the other arbobacteria. In animals, too, vaccine development is difficult. Thus, the vaccination has not been generally used against *Afipia (Bartonella) felis* in cats or against *C. burnetii* in sheep.

Vaccines against *R. rickettsii* were developed and also tested in Germany before and during World War II, above all for military interests, but could not reach pre-marketing stage due to inadequate protectiveness and considerable adverse effects [1] so that no vaccine is available today. Proteins influencing pathogenicity in *F. tularensis* infections have been successful in early vaccination attempts [89]. A live vaccine of *F. tularensis* with a deleted protein, which influences purin metabolism, creates immunity in mice protecting them from a deadly dose of the wild-type bacterium [90]. Partial immunity against *Y. pestis* with a vaccine consisting of Cafl protein can be created in mice [83].

7.1.3 Severity and Course of the Disease

About two thirds of the infections with arbobacteria have an asymptomatic and self-limiting outcome in immunocompetent individuals (see above). As the example of *R. rickettsii* shows, in case of clinical manifestation, a mild outcome and survival of the recipient is possible in case of early treatment (4th day), whereas the donor died without treatment [77]. Chronic arbobacteria infections manifest themselves with the usual signs of inflammation caused by bacterial toxins such as irregular febrile episodes, night sweat, exhaustion, fatigue, lymphatic adenopathy, arthritis of the small and large joints up to perivasculitis, endocarditis, myocarditis and encephalitis. Tick, louse and flea bites cannot always be identified from the patient's history. Since treatment is effective and without any major adverse effect, rapid antibiotic treatment should be started in case of suspected infection, even without specific pathogen identification; rapid treatment influences the survival rate, particularly in case of *Y. pestis* infection.

7.1.4 Therapy and Prophylaxis

7.1.4.1 Therapy

In case of unknown arbobacterium, doxycycline is the treatment of choice. Chloramphenicol, ampicilline, and cephalosporine are also very effective. As a rule, quinolone is effective in high dosages (750 mg twice daily for 5–7 days). Quinolone is only partly effective against *Rickettsia*. For *Yersinia*, a combination therapy with doxycycline and gentamicine should currently be the most effective one.

Since most pathogens, except for *Y. pestis,* grow intracellularly, treatment should continue for 2–3 weeks. Administration for a longer period is recommended if the nervous system is involved. Repeated infection, e.g. with *Borrelia*, will create partial immunity so that the *Borrelia* pathogens can be inactivated or eliminated via the immune system in case of re-infection without the occurrence of clinical symptoms.

7.1.4.2 Prophylaxis

In line with the main route of transmission, exposure to ticks and other vectors listed in Section 1.1 should be avoided in order to prevent infection. The pathogen can be transmitted by direct contact with *Bartonella, Coxiella, Francisella* and

Yersinia. Contaminations with these bacteria have also occurred in the laboratory.

7.1.5 Transmissibility

In principle, arbobacteria can be transmitted by a vector (for an exception, see 7.1.4.2). All arbobacteria at least temporarily infect endothelial cells, macrophages and neutrophil granulocytes, and can cause temporary bacteriaemia. They are transmissible via blood and/or erythrocyte and thrombocyte concentrates, especially in the acute initial stage of the infection. However, infections by arbobacteria have so far not been reported in Germany.

7. 1.6 Frequency of Administration, Type and Amount of Blood Products

Up to now, no transmission of arbobacteria via the blood which would be possible at the acute and the chronic stage of the infection has become known in Germany. Since the disease leads to signs of inflammation and to donor exclusion in compliance with the general guidelines, a large number of undiagnosed and unregistered transmissions should not be expected.

Transmission of arbobacteria via fresh frozen plasma is theoretically possible. Transmission by plasma derivatives can be excluded thanks to the manufacturing process since the bacteria are eliminated and inactivated entirely. Both heat inactivation and detergent treatment of plasma components will lead to loss of infectivity of the arbobacteria.

7.2 Blood Products

7. 2.1 Infectious Load of the Starting Material and Test Methods

Burden of blood or plasma with arbobacteria is unknown, but is to be considered as extremely low in Germany for reasons of infection epidemiology. For bacteria transmissible only by ticks, an accumulation of infections, if any, should only be seasonal; however, this has so far not been observed.

Since all arbobacteria also grow intracellularly and occur extracellulary, they cannot be removed from blood or cell-containing blood components.

7.2.2 Methods for Removal and Inactivation of the Infectious Agent

7.2.2.1 Separation

Arbobacteria cannot be removed from blood or cell-containing blood components. Leukocyte depletion can lead to pathogen reduction, e.g. for *Ehrlichia, Francisella* or *Rickettsia*, but cannot lead to complete removal.

In principle, bacteria can be removed from plasma by high revolution centrifugation or filtration over 0.22 µm filters. Neither method is feasible for fresh frozen plasma. Small bacteria such as *Coxiella, Rickettsia* and other arbobacteria types, but not *Borrelia* and *Yersinia*, can also penetrate filters of that pore size [2].

7.2.2.2 Inactivation

Arbobacteria can be inactivated by heat treatment >65 °C for 10 h or by pasteurising, e.g. at 60 °C for 10 h. For Gram-negative bacteria, lipopolysaccharide is released from the cell wall by heat treatment. If administered i.v., lipopolysaccharide can lead to febrile reaction up to endotoxin shock.

In thrombocyte concentrates, arbobacteria can be inactivated by treatment with psoralen or similar substances.

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

Arbobacteria can partly be replicated in cell culture to achieve quantities which allow spiking of blood or plasma. For *F. tularensis* ssp. tularensis, biosafety level 3 is required in compliance with the regulations for biological substances (Biostoff-Verordnung), safety level 2 is required for *Anaplasma, Bartonella, Borrellia* and *Ehrlichia*.

Validation of the procedures for epidemiological reasons is currently not necessary, especially since no arbobacteria infection by blood transmission has become known.

7.3 Assessment

Arbobacteria are pathogens which grow intracellularly and can replicate also in endothelial cells and macrophages or granulocytes, and are transmissible by blood. If transmitted, the pathogens can cause severe and even deadly infections. If the course is chronic, the disease can be consuming with organ function impairment.

Prevalence of arbobacteria infections is very low in Germany, except for *B. burgdorferi, B afzelii* and *B. garinii* infections, which can reach a prevalence of 10–30%, particularly in endemic areas.

The generally low risk of transmission of arbobacteria can be further prevented if donors with reduced hygienic status are excluded from donation. Acutely and chronically infected blood donors are recorded by their clinical symptoms and by general donor exclusion criteria, and the exclusion criteria have proved to be effective.

The low significance of arbobacteria transmission is proved by the fact that no transfusion-related transmission has been reported in Germany in the past 30 years. Arbobacteria have currently no significance for the safety of blood products and plasma derivatives in Germany.

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