Chapter 1

General introduction

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GENERAL INTRODUCTION

*Bartonella (B.) henselae* is the causative agent of cat-scratch disease (CSD). Classical CSD manifests as a prolonged painless swelling of a single lymph node mostly seen in children and young adults. In atypical cases CSD can invade other organs such as eye, liver, brain and bone. Due to improvement of diagnostic techniques, the insight in the spectrum of clinical disease resulting from infection with *B. henselae* is expanding. As both classical and atypical clinical presentations can mimic malignancies and infectious diseases such as tuberculosis, reliable diagnosis of CSD is important. This general introduction outlines the current knowledge regarding history, microbiology, clinical manifestations, diagnostic techniques, and treatment of *B. henselae* infection and gives an outline of the following chapters.

HISTORY AND MICROBIOLOGY

Although the clinical presentation of cat-scratch disease has been known since the early 1930’s, it took more than 50 years to isolate the causative bacterium. In 1931 cat-scratch disease was first recognised as ‘la maladie des griffes de chat’ in a 10-year old boy in Paris [1]. This boy, who had been scratched by many cats and subsequently developed enlarged lymph nodes, was primarily thought to have tuberculosis. Surprisingly his lymphadenopathy disappeared spontaneously, after which the association with cat scratches was postulated. In 1950 Debré reported about this and other patients with self-limiting regional lymphadenopathy in relation with cat contact. Around that time skin tests prepared from pus from CSD lymph nodes were developed allowing the identification of new patients [2]. In the following decades there were many reports about new CSD patients, resulting in hundreds of publications [3]. The broad spectrum of clinical presentations of CSD became more clear.

*Bartonella henselae*

The first convincing evidence of an infectious cause of CSD came in 1983 when Wear et al. demonstrated small pleomorphic bacilli in lymph nodes of CSD patients using the Warthin-Starry silver impregnation stain [4]. Their results were reproduced by others [5-8]. In 1988, English et al. reported the isolation and culture from the lymph nodes of CSD patients of the ‘cat-scratch disease bacillus’ [9]. This bacillus was later named *Afipia felis*, after the Armed Forces Institute of Pathology, where the organism was discovered [10]. However, the role of *A. felis* remained controversial for several years. Ultimately, it was found not to be the cause of CSD.

The causative agent of CSD, *Bartonella henselae*, was first identified in patients with AIDS with no history of CSD [11-13]. In November 1990, a single issue of the *New England Journal of Medicine* highlighted 3 different reports on the discovery of the same pathogen. Relman et al. used 16S rRNA-based polymerase chain reaction (PCR) to identify bacteria not yielded by culture in a patient with bacillary anigiomatisis [14]. Slater et al. grew a fastidious gram-negative bacterium in specimens from febrile patients with AIDS [15].
And finally, Perkocha et al. identified Whartin-Starry positive bacteria in a biopsy specimen from a patient with peliosis hepatis and AIDS [16,17]. These bacilli, formerly named *Rochalimea henselae*, appeared to be related to *Bartonella quintana* and *Bartonella bacilliformis*, that respectively cause trench fever and Carrion’s disease (also known as verruga peruana) [18].

The genus *Bartonella* includes 19 distinct species, of which at least 4 are responsible for human disease, as illustrated in Table 1. *B. henselae* is a small (0.6-1.0 μm) gram-negative oxidase-negative, fastidious, aerobic, rod-shaped, slow-growing bacterium. The novel species name, *Bartonella henselae*, was proposed in 1992 [19]. *Bartonella* is named after the Peruvian...
microbiologist Alberto Barton, who discovered *Bartonella bacilliformis* in 1905. The species *B. henselae* was named after Diane Hensel, who contributed to the initial isolation of the species [19].

Evidence that this bacterium was the cause of CSD in immunocompetent patients first came from serological studies [20,21]. In skin-test positive CSD patients, antibodies to *B. henselae* but not to *A. felis* were identified [22-24]. In 1993, Dolan et al. reported the first isolation of *B. henselae* CSD patients with typical lymphadenopathy [25]. Bergmans et al. identified *Bartonella* deoxyribonucleic acid (DNA) in pus aspirates of lymph nodes of CSD patients, while *A. felis* DNA was detected in none of them [26]. Nowadays it is generally accepted that CSD is caused by *B. henselae* infection.

An important clinical study of patients with CSD has been undertaken by Carithers, who reported over 1200 cases seen in pediatric practice [27]. It was recognized that *B. henselae* infection also causes atypical presentations of CSD in both immunocompromised and immunocompetent patients [28,29]. As diagnostic techniques have improved, the knowledge of the spectrum of clinical disease resulting from infection with *B. henselae* infections is still expanding.

Figure 2. Verruga peruana, caused by *Bartonella bacilliformis*, characterised by cutaneous eruptions of proliferated endothelial cells, often accompanied by bleeding of the lesions [205].
Table 1.
Bartonella species causing human disease.

<table>
<thead>
<tr>
<th>Disease in immunocompetent humans</th>
<th>Disease in immunocompromised patients</th>
<th>Reservoir</th>
<th>Vector</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. henselae</strong></td>
<td>Cat-scratch disease</td>
<td>Bacillary angiomatosis, Peliosis hepatis, Endocarditis, Persistent/relapsing bacteraemia with fever</td>
<td>Cats (mostly kittens), asymptomatic carriers</td>
<td>Cat-scratch, possibly cat flea</td>
</tr>
<tr>
<td></td>
<td>Atypical presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endocarditis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. bacilliformis</strong></td>
<td>Oraya fever, Verruga peruana, Asymptomatic carrier state</td>
<td>Unknown</td>
<td>Humans</td>
<td>Sandfly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. quintana</strong></td>
<td>Trench fever, Persistent / relapsing fever, Endocarditis, Asymptomatic carrier state</td>
<td>Bacillary angiomatosis, Peliosis hepatis, Endocarditis, Persistent/relapsing bacteraemia with fever</td>
<td>Humans</td>
<td>Human body louse</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>B. elizabethae</strong></td>
<td>Endocarditis</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

RESERVOIRS AND TRANSMISSION

Cats
Cats serve as the main natural reservoir for *B. henselae* [30,31]. Human CSD is directly linked to exposure to cats, especially young cats and cats with fleas [31]. Seroprevalence in pet cats ranges worldwide from low (1% in Sweden, 0-24% in different regions of Japan), to high (41% in UK and 44% in Italy) to very high (over 77% in the United States) [32-39]. The large differences in reported seroprevalence might partly be explained by differences in the selection of cats and the used serological techniques in the studies. The suggestion of an increasing gradient of seroprevalence in cats from cold climates to warm and humid climates is questionable. In The Netherlands seropositivity is reported as 56% for pet cats and 50% for cats in shelters [40].

Most infected cats seem to be healthy carriers of the bacterium with no clinical symptoms [41,42]. After transmission, *B. henselae* invades erythrocytes, evading the host immune response, resulting in high levels of bacteria in the feline host. The feline bacteraemia can persist for a year or longer [43]. *B. henselae* DNA was detected in the blood of 22% of the sheltered cats in The Netherlands [40]. Bacteraemia is more common in cats younger than 1 year of age (up to 56%) than in older cats (34%) and more common in stray cats than in pet cats [39-42].
Fleas
The cat flea (*Ctenocephalides felis*) accounts for cat-cat transmission. Bergmans et al. found *B. henselae* DNA in 26% of the fleas of sheltered cats [40]. The presence of cat fleas within the cat population is the risk factor most associated with high seroprevalence and is essential for the maintenance of the infection [44, 45]. The cat flea feces appears to play a crucial role in spread from cat to cat [46]. Other possible vectors have been suggested, such as ticks and flies [42, 47-49].

Other animals
There are some reports indicating other animals that may act as *B. henselae* reservoirs too. Dogs can be infected with *B. henselae*, often showing symptoms of disease [50]. Seroprevalence of *B. henselae* IgG is reported as 3% in the UK in healthy dogs and 10% and 35% in the United States (US) for healthy and sick dogs respectively [38, 50, 51]. Recently *B. henselae* DNA has been amplified in blood, lymph nodes and saliva of dogs [52, 53]. However, only rare cases of human CSD occur after exposure to a dog, presumably resulting from dog bites or from direct flea bites [53, 54]. *B. henselae* infection in other animals is reported sporadically, for example in wild animals such as lions, pumas and bobcats, but also in rodents and porpoises [55-60]. However, these other reservoirs seem to play no significant role in human CSD.
Humans

The primary mode of transmission of *B. henselae* from cats to humans is through a cutaneous trauma, usually caused by the scratch of a cat. How the bacilli reach the cat skin or nails is unknown. Transmission is less likely to occur by a cat lick or bite as shedding of *B. henselae* in cat saliva has never been clearly documented [61]. The possibility of direct transmission of *B. henselae* to humans by the cat flea is mainly hypothetical as it could not be proven experimentally [61]. However, patients likely to be infected by a tick bite have been reported, remarkably, with bacteraemia but not lymphadenopathy [62].

PATHOGENESIS AND IMMUNOLOGY

The role of the immune status

The nature and severity of the clinical presentation of *B. henselae* infection varies significantly with the immune status of the host [63,64]. Individuals with intact immune function usually are asymptomatic or present with classic CSD when infected with *B. henselae* [65]. In these cases infection is limited to skin and regional lymph nodes reflecting a strong cellular response to the bacterium [66]. Only in rare cases does the infection spread and become systemic in immunocompetent individuals.

In patients with impaired immune function, ‘immunocompromised hosts’, CSD often progresses to systemic disease. Those having acquired immunodeficiency syndrome (AIDS), immune suppression or other compromising health problems, tend to present with prolonged fever and vasoproliferation. The reduced ability of the immune system to control bacterial infection apparently results in a bacteraemia of longer duration and an altered *Bartonella*-host cell interaction [66]. This can lead to bacillary angiomatosis, which manifests as cutaneous angiogenic lesions consisting of vascular proliferation composed of endothelial cells and mixed inflammatory cell infiltrate. The mechanism by which *B. henselae* induces angiogenesis is not fully understood [67]. One hypothesis is that *B. henselae* induces secretion of proangiogenic cytokines by macrophages. Vascular endothelial growth factor (VEGF) induces proliferation of endothelial cells and angiogenesis [66,68]. Another hypothesis involves *Bartonella* directly triggering proliferation and apoptosis of endothelial cells, resulting in increased angiogenesis [69].

Angiogenic lesions are usually observed in highly immunocompromised persons with a low CD-4 count; often those infected with human immunodeficiency virus (HIV). Recently several reports are made on severe *B. henselae* infections in the emerging group of patients with immunosuppressive therapy. These reports concern mainly liver and kidney transplant recipients [70-75], but very recently similar observations have been made in patients receiving tumor necrosis factor-α (TNF-α) antagonist therapy or interferon-α and ribavirin for autoimmune disorders or chronic hepatitis C [76,77].
While the immune status clearly affects the severity and presentation of disease, there have been reports of systemic disease such as persistent bacteraemia, [78] endocarditis, [79,80] and bacillary angiomatosis [81] in immunocompetent patients. Conversely, classical CSD-like symptoms have been reported in patients with AIDS [82]. In otherwise healthy individuals, co-infections seem to influence the immune status resulting in more severe \textit{B. henselae} infection, illustrated by co-infections with \textit{Borrelia burgdorferi}, parvovirus and Epstein-Barr virus [48,83-86]. Furthermore, differences in virulence among various strains of \textit{Bartonella} may attribute to the variability in disease presentation [29].

\textbf{The immune response}

The immunopathogenesis of \textit{B. henselae} infection in humans remains poorly understood. Once transmitted to humans via cat scratch or bite, \textit{B. henselae} has an affinity for endothelial cells and is found in vessel walls, intracellular and free in necrotic debris [87]. Multiple cellular functions of the endothelial cells are subverted, resulting in cell invasion, proinflammatory activation, suppression of apoptosis, and stimulation of proliferation, which may cumulate in vasoproliferative growth [88]. Recently, a role for immune effector cells that produce angiogenic cytokines upon stimulation with \textit{B. henselae} has been proposed [66].

The histopathological response to infection with \textit{B. henselae} is granulomatous and suppurative. Early in the course of infection, lymphoid hyperplasia, arteriolar proliferation, and widened arteriolar walls are seen in biopsied lymph nodes. This progresses to granulomatous disease, with central areas of necrosis and multinucleated giant cells. The infection causes an interferon-\(\gamma\)-mediated T-helper cell response, resulting in macrophage recruitment and stimulation, ultimately producing granulomatous disease [89]. Later in the course of the disease, stellate microabcesses form with suppuration of affected lymph nodes [65]. In individuals with an intact immune system, infection generally remains within the lymphatics, with a symptomatic immune response that lasts 2 to 4 months [66].

In the feline host \textit{B. henselae} invades erythrocytes directly, but in humans it has been shown to invade CD-34+ hematopoietic progenitor cells instead [90]. Infection of these hematopoietic progenitor cells, that still are able to differentiate, results in intracellular presence and replication of \textit{B. henselae} in erythrocytes [90].

Cytokine and humoral response to \textit{B. henselae} infection have mainly been studied in cats and mouse-models [66,91,92]. Studies on the human immune response explaining the pathophysiological features of CSD, are rare. Levels of circulating Interleukin-2 (IL-2), IL-6 and IL-10 were significantly higher in patients with CSD than in healthy individuals, while no increases in IL-12, interferon-\(\gamma\) and IL-4 were detected [93].

Studies on human antibody kinetics revealed that seroconversion of immunoglobulin-M (IgM) and immunoglobulin-G (IgG) antibodies against \textit{B. henselae} occurs in most CSD patients [94]. Specific IgM is produced earlier than IgG after infection with \textit{B. henselae}. IgM mostly disappears in a few months, while IgG titers may remain positive for more than 2
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years [95,96]. B. henselae infection appears to confer lifelong immunity, as reports of recurrences of clinical CSD are extremely rare [65].

EPIDEMIOLOGY

Incidence

Epidemiologic studies from the United States, Europe, Israel, Australia, and Japan, have identified that CSD has a worldwide distribution [65,97]. The overall incidence of CSD is unknown because CSD is not a centrally reported disease. The true incidence is likely to be underestimated, as most cases do not need medical attention and only few cases require hospitalisation. A decade ago the incidence of CSD in the US was estimated at 4-10/100000/year, based on national databases [98,99]. Of 22000 reported CSD cases per year, over 2000 were being hospitalised annually in the US [98]. In The Netherlands, the estimated number of CSD patients was 2000 cases per year or 12.5 cases/100000/year, with an unknown hospitalisation rate [40]. The available data suggest that worldwide several thousands of cases of CSD may occur annually in many countries [42].

Cat-scratch disease occurs year-round but with a peak in fall and early winter, probably related to the seasonal birth of young kittens. It may occur in family clusters, with multiple siblings presenting simultaneously [100,101]. Carithers noted that there is significant asymptomatic infection in family members of CSD patients, and close contact with cats increased the prevalence of skin-test reactions [27]. CSD is slightly more common in males than in females [65].

It is believed that CSD is more common in children and young adults, based on series reporting up to 80% of CSD patients being younger than 21 years of age [27,29,98,102]. However, in other series the disease is common among adults (> 40% of patients being older than 20 years of age) but less likely to be recognised [31]. A surveillance study conducted in Israel showed that 6% of 846 immunocompetent patients with CSD were older than 60 years of age [103].

Seroprevalence

The reported seroprevalence of antibodies to B. henselae is highly variable [104]. Within Europe, IgG seroprevalence in healthy controls has been reported from 0% up to 66% [105,106]. These differences can be explained by several factors. First, geographical differences in climate and in local seroprevalence in cats can be of influence. Second, population factors like age, cat-ownership, and place of living (rural versus urban areas) appear to be important. Lastly, differences in serological assays and techniques, as well as different cut-offs and different interpretation of results may account for the wide variety in reported seroprevalence [104].
**Distribution of genotypes**

Two main genotypes of *B. henselae* have been identified in humans and cats, associated with CSD [107-110]. These two genotypes, genotype I (reference strain: Houston-1) and genotype II (reference strain: Marseille) show a world-wide distribution [42]. Migration of humans with their pets over the last few centuries account for the distribution in various parts of the world [42]. A recent report of the presence of *B. henselae* DNA in the teeth of cats buried in France between the XIIIth and XVIth centuries indicated the presence of mainly Houston-1, whereas in present times genotype II (Marseille) is highly predominant in French cats [111]. Some reports have emphasized that *B. henselae* genotype I is more commonly identified in human cases of CSD whereas genotype II is predominantly isolated within cat populations [112,113].

**CLINICAL MANIFESTATIONS**

Clinical presentation of *B. henselae* infection ranges from mild lymphadenopathy as in ‘classical CSD’, to more severe systemic involvement in ‘atypical CSD’ or ultimately to life-threatening systemic disease in the immunocompromised patient.

**Table 2.**

Signs and symptoms of *B. henselae* infection.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Local skin eruption (common), Erythema multiforma, erythema nodosum, thrombocytopenic purpura.</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Prolonged lymphadenopathy (common), suppuration 10%</td>
</tr>
<tr>
<td>General</td>
<td>Prolonged fever (common)</td>
</tr>
<tr>
<td>Internal organs</td>
<td>Granulomatous lesions in liver and/or spleen, glomerulonephritis</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Encephalopathy, convulsions, coma, myelitis, peripheral neuropathy</td>
</tr>
<tr>
<td>Eye</td>
<td>Parinaud’s oculoglandular syndrome, neuroretinitis, uveitis, iridocyclitis, glaucoma</td>
</tr>
<tr>
<td>Blood</td>
<td>Hemolytic anemia, thrombocytopenic purpura</td>
</tr>
<tr>
<td>Bones/joints</td>
<td>Arthritis, osteomyelitis</td>
</tr>
<tr>
<td>Heart</td>
<td>Endocarditis, myocarditis</td>
</tr>
</tbody>
</table>
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Classical CSD

For the purpose of this thesis, ‘classical CSD’ will refer to the most common, ‘typical’, presentation of infection with *B. henselae*. Classical CSD presents with the syndrome of a local skin lesion, and isolated lymphadenopathy, often accompanied with fever [3]. Early series noted prevalence of classical CSD in approximately 95% of CSD cases, but this is likely to be an overestimate, as many of the atypical presentations are only recently been recognized.

Dermatological manifestations

Three to ten days after the introduction of the organism into the skin by a cat-scratch, a cutaneous lesion develops at the primary inoculation site. Careful examination of the skin is often required to find this lesion. The lesion develops and generally evolves through vesicular, erythematous, and papular phases before it disappears, usually after one to three weeks (range several days to several months) [27,114]. In approximately 5% of cases inoculation does not occur at the skin but in the eye or mucous membranes. The inoculation lesions usually cause minimal symptoms and heal without scarring.

In rare cases the skin lesion evolves to a transient maculopapular and urticarial eruptions, erythema multiforme, erythema nodosum, or leukocytoclastic purpura [27,114,115].

![Figure 4. Vesicular *B. henselae* skin lesions at the site of the cat scratch](image)

Lymphadenopathy

The hallmark of cat-scratch disease is the regional lymphadenopathy appearing after approximately two weeks (ranging from 7 to 60 days) in the lymphatic drainage area of the inoculation. The lymph nodes are usually somewhat tender, with erythematous overlying skin and are occasionally suppurative (10-15%). Node size typically ranges from 1 to 5 cm, but may be as large as 10 cm [27,116]. The location of the lymphadenopathy is proximal of the site of the inoculation, most commonly in the axillary, cervical, and submandibular nodes and the groin. In 85% of cases lymphadenopathy is solitary or limited to one lymph drainage region [27]. Spreading to other regions may occur, while generalized lymphadenopathy is rare [114]. Lymphadenopathy associated with CSD usually resolves in one to four months, but
reports have described persistence of enlarged nodes for one to three years [27]. Systemic illness is mild in the majority of classical CSD patients, and can include fever, generalized aches, malaise, anorexia, nausea, and abdominal pain [27].

**Figure 5.**
Suppurative lymphadenitis in neck and axilla (arrows) of an eight-year old boy (A) and behind the ear of a seven-year-old girl (B), both with serologically proven CSD [207, 208].

**Atypical CSD**

In some CSD patients, *B. henselae* disseminates, potentially affecting nearly all organ systems. Involvement of liver and spleen, the eye and the central nervous system are most common. In the last 5 years over a hundred cases of atypical CSD have been reported in the medical literature.

**Hepatosplenic manifestations**

Involvement of the liver, spleen or both is one of the more common atypical manifestations of CSD [117-119]. The majority of the patients with visceral organ involvement have no accompanying peripheral lymph node enlargement. Patients may have persistent fever without apparent cause, as well as abdominal pain and/or weight loss [120]. About half of the children have hepatomegaly or splenomegaly. The liver may be tender to palpation. The erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) is typically elevated and liver function tests may be mildly abnormal. An abdominal computed tomography (CT) scan will typically show scattered, multiple defects in the liver and/or spleen that, if biopsied, show necrotizing granulomas. These lesions are manifested as hypo-echoic areas on ultrasound [121]. Biopsies are now rarely performed because the clinical and imaging findings are characteristic.
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Persistent fever without apparent cause

*B. henselae* infection can cause prolonged fever, and should be considered in patients with fever of unknown origin (FUO) [122,123]. FUO is commonly defined as fever lasting for > 2 weeks with no diagnostic signs or symptoms of an obvious clinical disease [67]. A prospective study showed that *B. henselae* was the third most common infectious disease diagnosis in 146 children with FUO [124]. In this study, the most common diagnoses were Epstein-Barr virus infection (15%), osteomyelitis (10%), *B. henselae* infection (5%) and urinary tract infection (4%). A history of cat exposure was not uniformly found among patients diagnosed with *B. henselae* infection. In different series focusing on patients with fever, one third to half of the patients with confirmed *B. henselae* infection presented with no symptoms of CSD (even absence of lymphadenopathy) other than fever [123-125].

**Ocular manifestations**

Ocular manifestations in atypical CSD are not rare. Best known is Parinaud’s oculoglandular syndrome, reported in 2-8% of patients with CSD. This syndrome is caused by inoculation near or in the eye, followed by granulomatous conjunctivitis and adjacent preauricular lymphadenopathy. Symptoms include foreign body sensation, unilateral eye redness, serous discharge and increased tear production. Parinaud’s oculoglandular syndrome is self-limiting, and seldomly leads to serious complications [7,126].

Other frequently reported ocular manifestations include neuroretinitis, panuveitis, papillitis, optic neuritis and retinochoroiditis [127]. Some authors believe *B. henselae* is the most common infectious cause of neuroretinitis, based on high *B. henselae* seroprevalence in neuroretinitis patients [128]. In certain populations, about 2-3% of all CSD patients may...
develop neuroretinitis, a syndrome of acute visual loss from optic nerve edema associated with macular exudates [129]. *B. henselae*-induced neuroretinitis typically presents with fever, malaise and unilateral blurred vision often associated with an afferent papillary defect [130]. Retinal findings may include hemorrhages, cotton wool spots, multiple discrete lesions in the deep retina and stellate macular exudates (known as a ‘macular star’) [130]. Development of a macular star may take 1-4 four weeks after initial symptoms, and can persist for months before resolving, sometimes leaving residual defects [127]. However, most patients with CSD neuroretinitis appear to have a good long-term prognosis [127,130].

**Figure 7.**
Fundus photograph of a ten-year-old boy showing *B. henselae* neuroretinitis with a macular star [210].

**Neurologic manifestations**
A wide range of neurologic manifestations are described in patients with CSD, including encephalopathy, meningitis, radiculitis and cerebellar ataxia [131]. Encephalopathy is the most common manifestation, typically presenting with abrupt confusion and disorientation, approximately one to six weeks after the initial presentation with lymphadenopathy. The majority of these patients will develop seizures and some develop coma or focal neurologic findings such as hemiparesis as a result of cerebral vasculitis [131,132]. Combative behaviour has been reported in as many as 40% of patients with *B. henselae* encephalopathy [133,134]. Diagnosis is difficult, as in most cases computed tomography (CT) scan of the brain is normal and pleiocytosis in the cerebrospinal fluid (CSF) is mild. Most patients have an abnormal electroencephalogram. Although patients typically recover from CSD encephalopathy within several weeks, some will have residual neurologic defects [131].

**Orthopedic manifestations**
In a recent study, one-tenth of 913 CSD patients showed musculoskeletal manifestations, mostly myalgia and arthropathy (arthralgia and/or arthritis). Tendinitis, neuralgia and osteomyelitis were far less common [135]. Severe and often disabling arthropathy can be prolonged [136]. In a surveillance study of 841 patients with CSD in Israel, 3% had rheumatoid factor-negative arthropathy. Knee, wrist, ankle and elbow joints were most frequently affected. Joint complaints resolved in the majority of patients [137].
B. henselae osteomyelitis, the most well known musculoskeletal manifestation, is in fact the rarest. It can occur in all different bones, sometimes leading to osteolytic lesions [136]. Vertebral involvement can cause severe neurological complications that are transient in most cases [138].

**Cardiac manifestations**

*B. henselae* endocarditis is reported many times, usually in patients with pre-existing cardiac valve lesions. Although most patients report cat contact, a history of CSD is often lacking for months to years in the past [139]. It can present as endocarditis alone or as part of systemic disease [81]. *B. henselae* myocarditis is probably rare [140,141].

**Other manifestations**

Manifestations of infection with *B. henselae* in organ systems not yet mentioned are rare. Hematologic complications include hemolytic anemia, thrombocytopenic purpura and coagulation disorders [142-144]. Renal disease has been reported, with glomerulonephritis being the most frequently encountered [68]. This immune complex-mediated disease is generally self-limiting, but can lead to renal failure [145]. Rare cases of pulmonary involvement have been reported, generally taking the form of pneumonia or pleural thickening and/or effusion [146]. Pulmonary manifestations appear 1 to 5 weeks after the appearance of lymphadenopathy and have a good prognosis.

*B. henselae* infection presenting as pseudomalignancy has been reported in the literature several times [147]. *B. henselae* infection mimicking lymphoma is one of the most frequently reported. The clinical presentation of lymphadenopathy with prolonged fever, night sweats, weight loss and intrasplenic lesions can be highly suggestive of lymphoma [148]. There are several reports in the literature presenting *B. henselae* infection as a solitary mass in the breast, mostly with axillary lymphadenopathy [149,150]. Simulation of various other tumors has been reported anecdotally [68].

*Figure 8.* Conventional radiographs of the left elbow showing bone lysis (arrows) due to *B. henselae* infection at the radial side of the distal humerus [211].
Immunocompromised hosts

Among immunocompromised patients, infection with *B. henselae* (as well as *B. quintana*) can cause a broad spectrum of systemic manifestations. This is most often described in HIV patients, but also reported in patients receiving immunosuppressive treatment. Immunosuppressed patients can present with classical CSD, but tend to have generalised disease.

These patients can present with lymphadenitis, splenitis, osteomyelitis, prolonged fever with bacteraemia and subacute endocarditis [16,17,151]. Other presentations are vasculoproliferative lesions such as bacillary angiomatosis and bacillary peliosis [15,17,152]. Bacillary angiomatosis is characterized by unique vascular lesions that are typically found on the skin, but can also involve regional lymph nodes, the respiratory tract, bones, gastrointestinal tract and central nervous system. The skin lesions are reddish-brown papules that may clinically resemble hemangioma, Kaposi's sarcoma and infectious lesions including tuberculosis and mycobacterial infections [152]. Patients may have accompanying symptoms including fever, chills, malaise, headache and anorexia with weight loss. Bacillary angiomatosis develops mainly in patients with HIV infection and severe immune suppression, especially those who have a CD-4 count of less than 100 cells/mm³ [153].

Bacillary peliosis is a specific form of *B. henselae* disease in the severely immunocompromised host. It is characterized by the development of characteristic dilated capillaries or blood-filled cystic structures. These lesions become several millimeters in diameter and typically occur in the liver, spleen or lymph nodes. Patients present with gastrointestinal symptoms, prolonged fever, chills and hepatosplenomegaly [120].

The incidence of *B. henselae* infection in immunocompromised patients is difficult to ascertain because they remain probably undiagnosed in a significant proportion of patients [154,155]. In South-Africa a 10% prevalence of *B. henselae* bacteraemia was found in 188 HIV patients, as determined by PCR [156]. Bacillary angiomatosis was reported to have a prevalence of 1.2 cases/1000 HIV patients in a German study [155]. In Greek HIV patients seroprevalence of *B. henselae* IgG antibodies was 41%, and of IgM antibodies 0.8% [157]. In The Netherlands the incidence of severe *B. henselae* infections in immunocompromised patients appears to be low, probably resulting from the consistent use of trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* pneumonia prophylaxis in most immunocompromised patients.

**DIAGNOSIS**

**Clinical criteria and skin test**

Formerly the diagnosis of CSD required the presence of three out of four criteria: contact with a cat resulting in a primary lesion, a positive skin test, regional lymphadenopathy and the presence of characteristic histopathologic features [27]. The skin test was based on a
delayed-type hypersensitivity response to intradermally injected heat-inactivated pus of a CSD patient. As the skin test was not specific, sensitive, nor safe when compared to new diagnostic tools, it is now considered obsolete [26,65,158]. There is still no diagnostic gold standard for the diagnosis of CSD. The diagnosis of CSD is based on typical clinical findings associated with exposure to cats and exclusion of other causes. Laboratory diagnosis of CSD is now based on serologic testing and/or polymerase chain reaction (PCR), because culture of the bacterium is difficult.

**General laboratory findings**
In CSD cases, haematological and clinical chemical laboratory findings are often non-specific. *B. henselae* infection may result in normal or mildly elevated white blood cell counts, and normal, elevated, or diminished platelet counts. Analysis of CSF typically yields normal results. Liver enzymes are usually normal and the ESR and CRP may be normal or elevated [68].

**Culture and histology**
Isolation of *B. henselae* from blood or tissue samples is difficult, as this gram-negative bacterium grows slowly and fastidiously. Even when employing optimal techniques with incubation for a minimum of 30 days, most blood cultures remain negative [13,42]. Histopathologic findings in the primary inoculation lesion and in affected lymph nodes are non-specific and depend on the stage of the disease. Development of granulomas and microabcesses are mostly present in affected tissues. Although non-specific, Warthin-Starry silver staining of pleomorphic bacilli within areas of necrosis would strongly suggest a diagnosis of CSD if observed in combination with typical clinical findings.

**Serology**
Indirect fluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA), are the most widely used tests for serologic diagnosis of CSD [43,159]. In the international literature elevated anti-*B. henselae* IgG antibody titer $\geq 1:64$ is considered to indicate infection when patients are tested at least two to three weeks after a clinically suspected infection [42]. However, the value of IgG has been questioned as high seroprevalence and cross-reactivity seems to limit specificity [95].

A wide range of sensitivities and specificities has been reported depending on study population, definitions of CSD, as well as used materials and techniques [94,95,160]. Some IFA tests are commercially available, although many laboratories use in-house made tests. Serological testing in The Netherlands is mainly performed in the National Institute for Public Health and the Environment (Bilthoven) and the Laboratory for Medical Microbiology and Immunology of the St. Elisabeth Hospital (Tilburg). Questions arose on the reliability of test results using in-house and commercial ELISA and IFA tests, leading to several studies described in this thesis.
An ideal test is highly sensitive and specific, giving high predictive values of positive and negative test results. Specificity, defined as the percentage of the non-diseased (healthy) cases actually testing negative, is highly important in the diagnostic process of CSD as the differential diagnosis contains serious diseases such as lymph node cancer or tuberculosis. Low specificity implicates many false positive results, with the risk of incorrectly diagnosing CSD, which may lead to delayed treatment of malignancies or serious infections. Sensitivity, defined as the percentage of diseased cases actually testing positive, is of importance when using a test as a screening tool [161]. Predictive values of positive and negative results are not intrinsic to the test, but depend also on the prevalence of the disease in the population [162].

Comparison of various serological tests from different laboratories is complicated by the lack of standardisation. Different types of IFA and ELISA tests have been described in differently defined patient groups and control groups. Sensitivity of IgM and IgG detection by different in-house prepared tests has been documented between 3-95% and 10-100% respectively, with specificity ranging from 64% to 100% [94,95,104,163]. Commercially available serological tests have not shown better results [163,164].

The optimal moment of serological sample taking in the course of disease has not been clearly established. Bergmans et al. demonstrated in 18 PCR positive CSD patients that IgM antibodies are detectable earlier than IgG, with disappearance of IgM within 100 days after onset of CSD and of IgG within 1 year in 75% of patients [95]. Metzkor-Cotter et al. confirmed disappearance of IgM positivity within 3 months in most patients, while IgG titers remained seropositive for >1 year after the onset of CSD in 25% of the patients. In some cases IgG remained detectable for >2 years after disease onset [96]. No association was found between antibody titers or their kinetics and the clinical manifestations or duration of disease. In immunocompromised patients, serological testing is thought to be less reliable, as HIV-infection and a low CD-4 count are associated with decreased ability to develop antibodies to Bartonella [165]. Therefore, in these patients testing must not be limited to serological tests to diagnose Bartonella infections in immunocompromised patients but should include PCR.

**PCR**

Polymerase chain reaction has been helpful in detecting the different Bartonella strains [166,167]. By means of this relatively new molecular biological technique, B. henselae DNA can be identified in patients’ tissue samples, pus and blood samples [168]. Due to the selection of a bacterium-specific target region, this technique is highly specific. Sensitivity of PCR is reported between 96% to 100% depending on the selected patient group and target regions used [26,95,169-172].

However, even PCR may have sensitivity and specificity problems, and the results rely heavily on the quality of the laboratory procedures [173]. Cross-contamination to subsequent samples by carryover of PCR products can be prevented by strict protocols.

The first described B. henselae PCR assay amplifies DNA coding for a 296 base pair fragment
of the 16S ribosomal RNA (rRNA) and later the 16S-23S rRNA intergenic regions of B. henselae species [174]. Recently, targeting on genes coding for proteins, such as citrate synthase (gltA), heat shock protein (groEL), riboflavine (ribC), RNA polymerase beta subunit (rpoB) or cell division protein (ftsZ) has proven to be useful in real time TaqMan PCR technology [167]. Real-time polymerase chain reaction is a relatively quick technique that uses amplification and simultaneous quantification of a specific sequence in a DNA sample.

If pus is not spontaneously released, invasive procedures to obtain tissue samples are needed for PCR. The timing of biopsy may influence the sensitivity of PCR, probably being highest in the first 6 weeks of CSD [175]. The use of blood samples for detection of bacterial DNA has proven to be useful in patients with Legionella pneumophila and Streptococcus pneumoniae infections [176]. The use of PCR in blood samples of CSD patients is only reported sporadically, and its value in clinical practice is not yet studied [168].

TREATMENT

The therapeutic approach to CSD varies on the basis of the clinical manifestations and immune status of the patient.

Classical CSD

Most patients with typical CSD have spontaneous gradual resolution of symptoms with no response to specific antibiotic therapy. The clinical manifestations of the disease may be due to an immunological reaction in the lymph nodes with few or no viable Bartonella bacilli by the time treatment is started [177]. This could explain why the bacterium is rarely isolated from lymph nodes in the acute phase of disease, and why the effect of antibiotic treatment is lacking [25,110].

Various reports tried to evaluate the effectiveness of different antimicrobial agents for the treatment of typical CSD, though only one randomised controlled trial has been conducted [178]. In vitro, the bacillus is susceptible to several antibacterial agents including penicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, quinolones, trimethoprim-sulfamethoxazole and rifampin. However, it is known that in vitro susceptibility does not correlate well with a clinical response [177].

Most investigators have observed no benefit of antibiotics in classical CSD, with only anecdotal reports suggesting that antibiotics may be effective in some cases. In an uncontrolled study of 83 CSD patients, Spaulding et al. noted the failure of any antibiotic to shorten the course of the illness [179]. Margileth et al. retrospectively reviewed the effects of various antibiotics in 268 CSD patients [180]. The mean duration of illness was 14.5 weeks for 179 patients not treated or treated with ineffective antibiotics, while the duration of illness was 2.8 weeks for 89 patients treated with rifampin, ciprofloxacin, gentamicin or trimethoprim-sulfamethoxazole.
Some smaller uncontrolled series have been reported. Holley et al. describes 5 adults with CSD and painful lymphadenitis showing rapid clinical improvement after oral ciprofloxacin [181]. Chia et al. describes 4 patients with localized CSD with a 50% reduction in lymph node size and complete resolution by day 5 and 14 respectively after receiving oral azithromycin for 5-10 days [182].

Bass et al. has reported the only prospective randomized double-blind placebo-controlled therapeutic trial. They examined the effectiveness of a five-day course of azithromycin in 29 immunocompetent children and adults with typical CSD [178]. An 80% decrease in lymph node volume (determined by ultrasound) during the first 30 days was seen in 7 of 14 patients who received azithromycin, compared to 1 of 15 placebo recipients (p = 0.026). However, after 30 days there was no significant difference in rate or degree of resolution between the two groups. Inherent to the small study population, no evidence was found for efficacy of azithromycin for the treatment or prevention of encephalitis, endocarditis or other severe sequelae.

In conclusion, there is little evidence to prove a clinically relevant benefit of antibiotic treatment in mild classical CSD, as untreated cases have a favourable outcome. The risk of adverse drug reactions and development of bacterial resistance supports the recommendation not to treat mild to moderately ill immunocompetent patients with CSD with antibiotics [177,183]. After exclusion of malignancies and mycobacterial or fungal infections, treatment should focus on reassuring the patients that the lymphadenopathy is benign and that it will probably subside spontaneously within 2 to 4 months. Management consists of treatment for pain with analgetics or needle aspiration in case of lymph node suppuration and prudent follow-up [184]. In more severe cases of CSD, antibiotic treatment can be considered. The preferred antibiotic agent is azithromycin, since this is the only one shown to be somewhat effective in a randomised controlled trial [178].

**Atypical CSD**

There are no controlled studies on treatment of atypical CSD. However, antibiotic treatment is recommended in patients with more serious manifestations of CSD, such as involvement of visceral organs, neuroretinitis, encephalopathy and endocarditis.

In case of hepatosplenic disease and prolonged fever due to *B. henselae* infection, a combination of rifampin and gentamicin or azithromycin for 10-14 days is suggested. As long as the patient improves, there is no need to repeat the imaging studies of the liver or spleen to document resolution of the lesions [120,177,178,185]. The optimal therapy for neuroretinitis is unknown. Based upon limited data, combination of doxycycline plus rifampin for 4-6 weeks in adults and rifampin plus azithromycin in children is suggested. Neuroretinitis should be managed in conjunction with close monitoring by an ophthalmologist [130,186]. Furthermore, little is known about optimal therapy for neurologic manifestations of *B. henselae* infection. In severe cases, some suggest therapy with a combination of doxycycline plus rifampin for 10 to 14 days, as more experience exists with doxycycline than with azithromycin in treating central nervous system infections caused by other pathogens [177].
Because *B. henselae* endocarditis can be fatal, this condition is usually treated aggressively with antibiotics. However, no antibiotic regimen has been proven effective in the literature. Therapy should probably include an aminoglycoside prescribed for a minimum of two weeks [187]. Frequently, surgical treatment of the endocarditis is needed [188].

**Prognosis**

In immunocompetent patients with classical presentation, CSD is usually a benign, self-limiting process. Even in atypical cases with impressive symptoms, such as encephalopathy, it usually resolves without sequelae. Only two fatal cases in immunocompetent patients have been described in the literature. Both cases concern otherwise healthy children, presenting with neurological symptoms leading to death, with post-mortem diagnosis of *B. henselae* meningitis and encephalitis [189,190].

**Immunocompromised hosts**

In immunocompromised patients with angioproliferative diseases, fatal outcomes can occur without treatment. In contrast with immunocompetent patients, immunocompromised patients often show an impressive clinical response to antibiotic treatment. Although never tested in a controlled study, antibiotics are strongly recommended in these cases [152]. Overall, prolonged courses of tetracyclines, erythromycin, doxycycline, rifampin, azithromycin, or a combination of these antibiotics are effective and should be administered in these patients for at least six weeks and be continued for 4 to 6 months or longer in those who have relapses [184].

**Corticosteroids**

Margileth mentioned that oral corticosteroids were used as an adjunct to antibiotics in several patients with severe systemic disease with favourable responses [180]. However, other patients with CSD and neuroretinitis did not respond to corticosteroids. Reports on corticosteroid therapy are limited to single cases or small series. A total of nine CSD patients with eye involvement recovered after treatment with antibiotics and oral corticosteroids [191-193]. Two case reports of children with CSD encephalopathy illustrate that adding high-dose corticosteroid therapy might have a beneficial effect on recovery [194,195]. However, the natural dramatic improvement of severe neurological symptoms may falsely suggest that the steroids have influenced the course. Indeed, many cases of encephalopathy are known with favourable outcomes without steroid therapy.

In immunocompromised patients even more caution is warranted, as studies are lacking and steroids might further weaken the immune system. However, there is one case report of an 8-year old girl with methotrexate treatment for juvenile rheumatoide arthritis suffering CSD with very large lymph nodes. She recovered after oral prednisone was added to antibiotic treatment [196].

Until controlled studies have determined a positive effect of corticosteroid therapy on duration and severity of disease and long-term outcome, no specific recommendations can be made on corticosteroid therapy in CSD.
**Prevention**

In healthy persons, the risk of severe CSD is very low. Effective means of preventing *B. henselae* infection are hygiene and cautious cat-handling to prevent scratches, especially with young kittens. It is recommended to wash hands after handling pets and promptly clean any cuts, bites or scratches with soap and water [42]. Flea control is one of the major control measures to prevent cat infection and its spread from cat to cat [197]. Testing of cats for *B. henselae* infection is not recommended. A feline vaccine to prevent the spread of infection in cat populations and reduce human risk of infection is not yet available [198]. The effectiveness of such a vaccine can be hampered by lack of cross-protectivity between different genotypes [199].

In immunocompromised patients it is advised only to adopt cats raised in a ‘clean’, flea-controlled environment. In these patients, selection of seronegative cats can be considered, as those are more likely not to be bacteremic [41]. It has been shown that multiple cat ownership was associated with an increased risk for *Bartonella* infection [200].

The role of dogs as reservoirs and the role of ticks and fleas as possible vectors is not yet clear. However, prevention of tick infestation, direct removal of ticks as well as flea control may reduce the risk of infection [50,201].

**CONCLUSION**

Cat-scratch disease classically causes regional lymphadenopathy that is self-limiting in the majority of cases, but in atypical cases it can cause severe disease. On clinical grounds, both classical and atypical CSD can be difficult to differentiate from other infectious diseases and malignancies. Reliable confirmation of the diagnosis by laboratory testing is therefore of great importance. Reported sensitivity and specificity of the different techniques differ widely. The question arises whether the current serological techniques and PCR are reliable in clinical practice and whether they can be improved. Due to development of diagnostic techniques, the knowledge of the clinical spectrum of CSD is expanding. Atypical CSD has many manifestations that need further exploration.

**AIMS OF THIS THESIS**

The objective of this thesis was three-fold. The first aim was to evaluate current serological techniques for anti-*B. henselae* detection (IFA and ELISA) and to search for possibilities of improvement of these techniques. The use of different *B. henselae* strains in the tests, age dependency and cross-reactivity were taken into account. The second aim was to develop and evaluate a new real-time PCR and to find new applications in a clinical setting. The last aim
was contribute to the understanding of the clinical spectrum of CSD by investigating the role of *B. henselae* infection in the etiology of Henoch-Schönlein purpura and by describing and reviewing vertebral osteomyelitis, one of the atypical presentations of CSD.

**OUTLINE OF THE FOLLOWING CHAPTERS**

**Part II**

*B. henselae* serology for diagnosis of cat-scratch disease

In this part the performance of different serological techniques in diagnosing cat-scratch disease is addressed and the possible improvements of the techniques are evaluated. In Chapter 2 the value of anti-*B. henselae* IgM and IgG IFA and ELISA is studied in a group of CSD patients and controls. In Chapter 3 a *B. henselae* IgM ELISA is evaluated in a routine laboratory setting. In Chapter 4 different *B. henselae*-specific diagnostic models using IgM and IgG including patient’s age are analysed. In Chapter 5 different serological techniques are evaluated, including new commercial tests and a test containing both Houston and Marseille strains. Serological cross-reactivity is tested in samples of patients with other infectious diseases.

**Part III**

*B. henselae* PCR for diagnosis of cat-scratch disease

In Part III the use of a real-time PCR is evaluated. In Chapter 6 a real-time PCR targeting the *gro-EL* gene is validated in patients with suspected CSD. In Chapter 7 the use of this *gro-EL* real-time PCR is evaluated in blood samples of CSD patients.

**Part IV**

Clinical aspects of cat-scratch disease

Chapter 8 and 9 are focused on clinical aspects of CSD. In Chapter 8 a prospective study, evaluating the role of *B. henselae* infection in Henoch-Schönlein purpura is described. In Chapter 9 a case of vertebral osteomyelitis caused by *B. henselae* infection and review the literature on this atypical presentation of CSD is reported.

**Part V**

General discussion

In Chapter 10 a general discussion on the findings is provided. Lastly, English and Dutch summaries of the thesis are given.
References

General introduction


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Photograph retrieved from Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany; http://www.medizin.uni-tuebingen.de.


