SUMMARY

Part I
Introduction

In the introduction of this thesis (Chapter 1) an overview is given of the different aspects of cat-scratch disease (CSD) as described in the literature. History, microbiological aspects, epidemiology, immunology and pathogenesis are discussed. In addition to the mild classical presentation, CSD can present atypically with severe disease. An overview is given of the broad spectrum of clinical manifestations of infection with *B. henselae*. The importance of reliable laboratory diagnosis is noted, as both classical and atypical CSD can be difficult to differentiate from other infectious diseases and malignancies on clinical grounds. Laboratory diagnosis of CSD mostly relies on serology and polymerase chain reaction (PCR), as culturing of the bacterium is difficult and histological findings are not specific. A review is given of the literature on different diagnostic techniques and the current knowledge of treatment.

The aims of this thesis were to evaluate and improve current serological techniques and to evaluate the usefulness of testing serum samples by use of a new real-time PCR for CSD diagnosis. Moreover, we aimed to clarify the role of *B. henselae* infection in the etiology of Henoch Schönlein purpura and to review the literature on vertebral osteomyelitis, one of the atypical presentations of CSD.

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

In Chapter 2, the diagnostic value of five serological tests was studied in a group of 51 proven CSD patients and 55 negative controls. The diagnosis of CSD was based on clinical data, a positive PCR, and the lack of another diagnosis that might explain the symptoms. The controls were patients initially suspected of CSD but with another diagnosis and negative PCR. We concluded that testing for anti-*B. henselae* IgM (IFA and ELISA) had low sensitivity (53-65%) and that specificity (91-93%) was not ideal. Detection of anti-*B. henselae* IgG has limited clinical value in diagnosing acute CSD due to a low sensitivity and high seroprevalence in controls. Approximately two-thirds of the CSD patients were infected with genotype I and one-third with genotype II. Although not significant for all tests, sensitivity seemed to be higher in patients infected with genotype I than in those infected with genotype II. This might be explained by the use of genotype I strains in the tests. IgM sensitivity was highest within 6 weeks after onset of symptoms, with a sharp decrease after 8 weeks, while IgG
sensitivity peaked at 6-8 weeks and then slowly decreased. A commercial IgM IFA proved of no clinical value in our population.

In Chapter 3, *B. henselae* ELISA was evaluated in a routine laboratory setting in PCR-positive CSD patients (n = 126) and age-matched controls (n = 126). Lymphadenopathy was reported in 97% of CSD patients, with 3% of patients showing atypical CSD. Low sensitivity was found for anti-*B. henselae* IgM (56%) and IgG (36%) detection when cutoffs were set at a high specificity level (98%). Sensitivity of IgG testing was considered too low for practical use. Of 55 CSD patients with negative or inconclusive *B. henselae* serology, consecutive samples showed no seroconversion of antibodies in 82% of cases. We concluded that, despite low sensitivity, IgM detection still could be useful in a clinical setting, although negative IgM does not exclude CSD. Anti-*B. henselae* IgM seroprevalence peaked in fall and winter and was highest in the 10-20 year age group. Relatively high IgM and IgG seroprevalences in a group of *B. henselae* PCR negative patients suggested that also a negative PCR does not exclude CSD. We concluded that the moment of sample collection influences the sensitivity level of both the serology and PCR test.

In Chapter 4, predictive models were designed to study the value of ELISA-based IgM and IgG detection in CSD and the influence of age on diagnostic performance. No age-dependency of IgM levels was found in CSD patients (n = 155) nor in controls (n = 244). However, with age, a highly significant increase of IgG response was found in controls. Analysis of several *B. henselae*-specific diagnostic models showed that IgM is a better predictor of CSD than IgG, with higher sensitivity when using both. Although the use of an age-factor further increased the sensitivity of IgM and IgG, it did not result in a clinically relevant increase of positive and negative predictive values (PPV and NPV) in the total population. However, in children, the use of IgM and IgG with an age-factor improved the PPV by 2% and the NPV by 8% compared to the use of IgM alone, which might be relevant in clinical practice.

In Chapter 5, seven different *B. henselae* serological techniques were evaluated in the CSD and control group that were described in Chapter 2. In this chapter, new commercial tests, including a new test containing the Marseille strain, were studied. Serological cross-reactivity was evaluated in 289 patients with other infectious diseases.

In both in-house and commercial tests, sensitivity of anti-*B. henselae* IgM detection was lower than that for IgG detection. We showed that combining the results of IgM and IgG detection, compared to IgM alone, improved sensitivity but lowered specificity in CSD diagnosis. The reduction of specificity, which was not statistically significant in this study, may be clinically significant. The Houston-strain (genotype I) tests were compared to a new IFA test containing Marseille strain (genotype II). The additional use of the Marseille strain IFA did not improve sensitivity or specificity and resulted in higher cross-reactivity. In fact, sensitivity of the Marseille strain test was lower in Marseille strain infected patients than in those infected with the Houston strain. In samples of patients with infectious lymph node diseases (i.e. Epstein-Barr virus, cytomegalovirus, *Toxoplasma gondii*, and *Streptococcus pyogenes*), cross-reactivity in *B. henselae* serology varied per test and disease: 0-5% in 5 IgM tests and 0-8% in an IgG test. Cross-reactivity also differed between tests and diseases and proved to be highest (13-30%) in sera of *Coxiella burnetii* patients. We concluded that the Marseille
strain test was of no additional value and that cross-reactivity should be taken into account when interpreting CSD serology.

Part III

B. henselae PCR for diagnosis of cat-scratch disease

In Chapter 6, an in-house real-time PCR targeting the groEL gene was validated in patients with suspected CSD. This new PCR showed 100% agreement with the conventional 16S rDNA-based PCR in seventy-three clinical samples. Testing was only positive for several Bartonella strains, while negative in many other bacteria. We concluded that this real-time PCR proves to be a sensitive, specific and quick method that is suitable for routine CSD diagnostics.

In Chapter 7, real-time groEL PCR was used for the detection of B. henselae DNA in serum samples of proven CSD patients. In a few cases (3/18), DNA was detectable in serum samples of CSD patients, but sensitivity seems too low for clinical diagnosis. In a small number of full blood and plasma samples of CSD patients, no B. henselae DNA was found.

Part IV

Clinical aspects of cat-scratch disease

In Chapter 8, we studied the role of B. henselae infection in Henoch Schönlein purpura (HSP). We compared B. henselae antibodies in forty-five HSP patients and ninety, age and gender matched, controls. Anti-B. henselae IgM was not detectable in HSP patients, and IgG seroprevalence was not higher in HSP patients than in controls. No B. henselae DNA was found in the acute phase serum samples. We found no evidence for acute or earlier B. henselae infection in HSP patients, which is in contrast to earlier reports in the literature. Also, no difference in seroprevalence of streptococcal antibodies was found between patients and controls. We concluded that B. henselae infection plays no significant etiological role in HSP.

In Chapter 9, we reported a case of vertebral osteomyelitis caused by B. henselae and reviewed the literature on this atypical presentation of CSD. We concluded that B. henselae vertebral osteomyelitis is a rare disease, mostly presenting with fever and sometimes with neurological symptoms. Most cases were treated with antibiotics and some required neurosurgical treatment. Generally, the prognosis of B. henselae vertebral osteomyelitis was good.
Part V
General discussion and summary

In chapter 10 the findings of this thesis are discussed and recommendations for future studies are made.