Chapter 2

Serological testing for *Bartonella henselae* infections in The Netherlands: clinical evaluation of immunofluorescence assay and ELISA

MJ Vermeulen
M Herremans
H Verbakel
AM Bergmans
JJ Roord
PJ van Dijken
MF Peeters

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ABSTRACT

Cat-scratch disease (CSD), caused by *Bartonella henselae* infection, can mimic malignancy and can manifest atypically. Therefore reliable serological testing is of great clinical importance. In this study the diagnostic performance of immunofluorescence assay (IFA) and ELISA was evaluated in a group of Dutch patients with proven CSD (clinical diagnosis confirmed by PCR). Sera of 51 CSD patients and 56 controls (similar symptoms, but other confirmed diagnosis and negative PCR for *Bartonella henselae*) were tested for anti-*Bartonella henselae* IgM and IgG by IFA and ELISA. A commercially available IFA test for IgM (Focus Diagnostics) performed poor with a sensitivity of 6%. The in-house assays for IgM showed a specificity of 93% (IFA) and 91% (ELISA), with low sensitivities (53% and 65% respectively). With a specificity of 82% (IFA) and 91% (ELISA), in-house IgG testing showed a significantly higher sensitivity in IFA (67%) than in ELISA (28%, p < 0.01). Sensitivity was higher in genotype I (38-75%) than in genotype II (7-67%) infections, but this was only statistically significant for IgG ELISA (p < 0.05). In conclusion, detection of IgM antibodies against *Bartonella henselae* by in-house ELISA and IFA is highly confirmative for the diagnosis of CSD. The high seroprevalence in healthy individuals limits the clinical value of IgG detection for diagnosing CSD. Given the low sensitivity of the serological assays, negative serology does not rule out CSD and warrants further investigations including PCR of relevant material in patients with suspected CSD. Adding locally isolated (e.g. genotype II) *Bartonella henselae* strains to future tests might improve the sensitivity.
INTRODUCTION

*Bartonella henselae* (*B. henselae*) is the causative agent of cat-scratch disease (CSD), which usually presents as a self-limiting lymphadenopathy. In a minority of cases including immunocompromised hosts, *B. henselae* can cause atypical infections such as osteomyelitis, endocarditis or peliosis hepatitis. The prolonged painless lymphadenopathy may mimic malignancies and tuberculosis [1,2]. Quick and reliable confirmation of CSD can prevent unnecessary diagnostic procedures or reveal cases of CSD in which antibiotic treatment needs to be considered.

For over thirty years, diagnosis of CSD had relied on clinical criteria and skin tests [1]. Nowadays serology is usually the first step that clinicians use for the confirmation of suspected CSD because it is a simple procedure [3,4]. Indirect immunofluorescence (IFA) and enzyme-linked immunosorbent assay (ELISA) are used for detection of anti-*B. henselae* antibodies in serum. Although IFA is the most widely used technique, the test is more time consuming than ELISA and interpretation might be less objective [5]. Evaluation of serological tests in earlier studies reported various sensitivities and specificities depending on study population, definitions of CSD, as well as used materials and techniques [3,6,7].

The bacterium is difficult to culture from patients, but polymerase chain reaction (PCR) for detecting *B. henselae* DNA in pus or lymph node specimen is highly specific and sensitive. The sensitivity of PCR is dependent on the used target genes and on the selected patient group [3,8-12]. The disadvantage of the PCR method is the need for invasive sample taking of pus or other relevant tissue [10].

Although recent studies suggest a more complex classification of *B. henselae*, until recently two *B. henselae* 16S rRNA genotypes have been identified associated with CSD cases [13-15]. These genotype I (corresponding to serotype Houston-1) and genotype II (serotype Marseille) can be distinguished by PCR [16]. After discovery of genotype II, Drancourt et al. suggested influence of the genotypes on the accuracy of the serological tests, which has not yet been confirmed [13].

In this study the diagnostic performance of two serological techniques (IFA and ELISA) was evaluated in patients with a clinical presentation consistent with CSD confirmed by PCR and a clinically relevant control group with negative PCR. A commercial IFA was compared to the in-house assays. Additionally, the influence of two different *B. henselae* genotypes on the sensitivity of these serological assays was examined.
MATERIALS AND METHODS

Patient population

In The Netherlands, material from patients clinically suspected of \textit{B. henselae} infection is sent to two national reference laboratories, the National Institute for Public Health and the Environment in Bilthoven (RIVM) and to the Regional Public Health Laboratory in Tilburg (RPHL). Patients who had material sent in for both \textit{B. henselae} PCR and serology, with enough serum stored for additional testing were selected from the laboratory databases of both centres. The referring physicians were asked to fill in an anonymised form with regards to clinical and epidemiological data (age, gender, symptoms of disease, duration of illness, cat contact and final diagnosis). As shown in Figure 1, patients were divided into the study group (CSD group) and the negative control group, according to clinical data and PCR results.

CSD group: patients with a clinical presentation of CSD based on retrospective analysis of clinical data and a positive PCR test for \textit{B. henselae}. Clinical presentation of CSD was defined as lymphadenitis or an atypical presentation of \textit{B. henselae} infection and absence of another diagnosis. The combination of matching clinical data and a positive PCR result was considered the golden standard for a proven infection with \textit{B. henselae}.

Control group: patients whose material was sent for \textit{B. henselae} testing, but eventually had a different clinical diagnosis (Table 1) and a negative \textit{B. henselae} PCR result.

Exclusion: patients not meeting the criteria for the two groups or with insufficient clinical information about their diagnoses were excluded.

![Figure 1. Diagram showing total patient number initially suspected of cat-scratch disease (CSD) subdivided based on clinical analysis and \textit{Bartonella henselae} PCR, resulting in three groups: CSD group, control group and excluded patients.](image-url)
Laboratory techniques

All serum samples were analysed for *B. henselae* specific IgM and IgG antibodies by ELISA technique at the RIVM and by IFA at the RPHL. If two or more serum samples from one patient were obtained (n = 16), the specimen collected nearest to the date of collection of PCR material was analysed. Sera were stored at minus 20 °C.

**Immunofluorescence assay**

In-house antigen slides for detection of IgM and IgG antibodies to *B. henselae* were processed using a method described earlier [3]. A bacterial suspension of approximately 108 organisms/ml was made from *B. henselae* ATCC 49882 (*B. henselae* type Houston-1), grown on Columbia agar supplemented with 5% sheep’s blood. The suspension was mixed with 50 μl/ml egg yolk emulsion [Unipath Ltd. Basingstoke, Hampshire, England], and spotted onto Teflon-coated slides. The slides were air dried for 30 minutes and fixed in acetone for 30 minutes. Sera and conjugates were diluted in phosphate-buffered saline (PBS; pH 7.2) containing 0.05% bovine serum albumin (BSA) and 0.1% NaN3. For detection of IgM antibodies, sera were pre-treated with IgG blocking solution [Incstar Corporation, Stillwater, Minnesota, USA].

The commercially obtained slides for detection of *B. henselae* IgM [Focus Technologies, Cypress, USA, Product Code IF1300M] are marketed for in vitro diagnostic use (only) outside the USA. The commercial slides were used simultaneously with the in-house prepared slides according to the manufacturers recommendations.

All IFA results were evaluated independently by two experienced operators, who were blinded to the clinical and laboratory findings. Interobserver differences never exceeded one dilution step. In case of disagreement (n = 11), the opinion of an independent third operator was conclusive. The most frequently reported cut-offs in the literature, namely positivity at titers of ≥ 1:64 for IgG and ≥ 1:8 for IgM were used [17].

**ELISA**

Detection of anti-*B. henselae* IgM and IgG by an in-house ELISA was performed based on the methods described previously by Bergmans et al. [3] and Barka et al. [18] with several modifications to improve sensitivity. The *B. henselae* strain ATCC 49882 was grown on Columbia agar plates containing 5% sheep’s blood for 7-10 days at 35 °C in an atmosphere containing 5% CO2. Colonies were scraped from the plates, suspended in PBS, sonificated for 30 minutes and stored at -20 °C. Wells of microtiter plates [Nunc, Polysorb] were coated overnight at +4 °C with 100 μl of an optimal dilution of the prepared *B. henselae* antigen in PBS. Parallel wells were coated with PBS alone to control for non-specific binding of serum components. After incubation the plates were washed twice with PBS with 0.05% Tween20 and blocked with blocking reagent [Boehringer, cat no. 1112.589] according to the manufacturer’s instruction. Serum dilutions (1:100) were added and then incubated for 1 hour at 37 °C. Plates were washed four times between each incubation step. For the IgM assay, prior to testing, sera were depleted of IgG with IgG-RF sorbent [Biotest, cat no. 807799], according to the
manufacturer’s instruction to prevent possible inter-isotype competition. Bound antibody was detected by horseradish-peroxidase labeled goat anti-human IgM [Biorad 75061] or rabbit anti-human IgG [Dako P021402] (1 hour, 37 °C). Tetramethylbenzidin (TMB) was used as substrate, and colour development was stopped after 10 minutes by the addition of 2M H2SO4. The plates were read at 450 nm (second filter 690 nm). A high positive, low positive and negative control serum were examined in each assay. In order to create a highly specific test, a total of 126 age-matched controls to a group of PCR positive CSD patients were used to calculate optimal cut-off values of the IgM and IgG ELISA’s. Cut-off values were set at the average optical density (OD) in this group plus 3 times the standard deviation (SD) for IgM and the average plus 2 SD for IgG to reach acceptable specificity levels, of 97% and 98% for IgM and IgG respectively. A serum sample was considered positive if the calculated ratio (OD sample/cut-off value) was ≥ 1, which corresponded to a cut-off ≥ 0.110 OD and 0.623 OD for IgM and IgG respectively.

PCR
Pus aspirates and biopsy specimens from lymph nodes were used for PCR detection of \textit{B. henselae} DNA with primers based on the 16S rRNA gene. This test has been described by Bergmans et al. with a sensitivity of 96% in skin test-positive patients [10]. If the \textit{B. henselae} PCR was positive, a 16S rRNA genotype-specific PCR was run to detect type I and type II genotypes as described earlier [16].

Data analysis
The data were analysed using SPSS 11.0 for Windows. Clinical and epidemiological data of the CSD and the control group were compared by use of the two-sample t-test or, when the normal distribution assumption was not met, the Mann-Whitney-U-test for continuous data, and the Chi-square test for nominal data. Sensitivity and specificity were calculated for detection of IgG and IgM in the IFA and the ELISA test. Receiver Operating Characteristic (ROC) curves were generated for the different tests, with the area under the curve (AUC) indicating the measure of accuracy. As IFA results are expressed as titers, only a select amount of cut-offs were possible. Samples were tested only at a 1:8 dilution for IgM, and at 1:8, 1:16, 1:32 and 1:64 for IgG. Therefore, the ROC curve of IFA test is angular in contrast to the gradual line of the ELISA with its numerical results. Subsequently, comparing the areas under the curve of IFA and ELISA is not entirely appropriate for evaluation of the value of ELISA and IFA for testing IgM and IgG. In order to compare the two tests, the specificity of ELISA was levelled with that of IFA. Therefore the cut-off of ELISA tests was changed to attain the specificity of IFA at cut-off 1:8 (IgM) and 1:64 (IgG). Comparison of the sensitivity of both tests was then possible at equal specificity by use of McNemar’s test for matched pairs for IgM and IgG. Sensitivity of serological testing was compared for different \textit{B. henselae} genotypes. Group differences were considered to be statistically significant if their two-tailed p-values were 0.05 or less.
RESULTS

Patient data

From January 2000 until June 2001, 171 patients were selected with a 86% response on the clinical data enquiry. According to the clinical data and PCR results these patients were divided into the CSD group and the negative control group (Figure 1). Sixty-four patients were excluded with no or insufficient clinical data (n = 23), undefined diagnoses (n = 13) and patients with clinical presentation of CSD but a negative PCR result (n = 28).

CSD group

This group (n = 51) consisted of patients with a clinical presentation of CSD with a positive PCR test for *B. henselae*. The median age of the CSD patients was 27.0 years (range 1-83), which was not significantly different from the control group (median 31.2 years, range 1-81). No statistically significant difference was found in gender between the CSD group (71% male) and the control group (57% male). In the CSD group most patients (96%) presented as typical CSD with

### Table 1.
Clinical diagnoses in the control group (n = 56).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients (%)</th>
</tr>
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<tbody>
<tr>
<td>Infection</td>
<td>20 (36 %)</td>
</tr>
<tr>
<td>Mycobacterial (atypical)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Mycobacterial (typical)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Bacterial, other</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>Viral</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>21 (38 %)</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>other</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Immunologic disorder *</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Congenital cyst/fistula</td>
<td>5 (8.9%)</td>
</tr>
<tr>
<td>Other diagnosis</td>
<td>2 (3.6%)</td>
</tr>
</tbody>
</table>

* Reactive lymphadenitis in Human Immunodeficiency Virus infection, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, autoimmune lymphoproliferative syndrome and Devic’s disease.
lymphadenitis. In 45% of these cases the lymphadenitis was axillary, and 31% in the neck region. Two patients presented with atypical CSD (multifocal osteomyelitis and endocarditis) without lymphadenitis. Information about cat contact was available in 33 CSD cases, with 88% reporting cat contact in the 3 months prior to beginning of symptoms and 12% reporting no cat contact.

Control group
Patients in the control group (n = 56) were diagnosed with malignancies (38%), with infections other than CSD (36%) and with other clinical diagnoses (26%) (Table 1). In the control group 87% of the patients had lymphadenopathy with the neck region being the most frequent localisation (59%).

The time interval between collection of PCR and serum samples was less than 1 week in 47% of the cases and more than 10 weeks in 21% of the cases, which was not significantly different for the CSD and the control group. To rule out the influence of a large time interval, data were reanalysed excluding the cases with a time interval longer than 60 days (n = 18), which did not change the results (data not shown).

Serology results

Test results and predictive values are shown in Table 2.

Table 2.
Serological test results for B. henselae IFA and ELISA serology for cat-scratch disease (CSD) patients and the control group; positive and negative predictive values.

<table>
<thead>
<tr>
<th></th>
<th>IFA commercial</th>
<th>IFA in-house</th>
<th>ELISA in-house</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>CSD group n=51 (n, %)</td>
<td>3 (6)</td>
<td>48 (94)</td>
<td>27 (53)</td>
</tr>
<tr>
<td></td>
<td>34 (67)</td>
<td>17 (33)</td>
<td>33 (65)</td>
</tr>
<tr>
<td></td>
<td>14 (28)</td>
<td>37 (73)</td>
<td></td>
</tr>
<tr>
<td>Control Group n=56 (n, %)</td>
<td>0 (0)</td>
<td>56 (100)</td>
<td>4 (7)</td>
</tr>
<tr>
<td></td>
<td>10 (18)</td>
<td>46 (82)</td>
<td>5 (9)</td>
</tr>
<tr>
<td></td>
<td>5 (9)</td>
<td>51 (91)</td>
<td></td>
</tr>
<tr>
<td>Predictive value (%)</td>
<td>100</td>
<td>54</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>73</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>58</td>
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</tbody>
</table>
In-house IFA
With the in-house IFA a specificity of 93% for IgM and 82% for IgG was reached with a sensitivity of 53% and 67% respectively. Combining in-house IgM and IgG (defining positivity as a positive IgM and/or a positive IgG) increased sensitivity to 86%, at the expense of specificity reduction to 77%.

Commercial IFA
Three of the 51 CSD patients tested positive with the commercial IFA IgM slides corresponding to a sensitivity of 6%. All samples of the control group tested negative (specificity 100%).

In-house ELISA
The ELISA showed a specificity of 91% for both IgM and IgG and a sensitivity of 65% and 28% for IgM and IgG respectively. Combining IgM and IgG results in the ELISA improved sensitivity (77%) while reducing specificity (82%).

Duration of disease
Of 41 patients (44 sera) in the CSD group the date of first clinical symptoms was known. The duration of disease at the moment of blood sampling did not significantly influence the sensitivity nor specificity, except for IFA IgM showing a better sensitivity for blood samples taken within six weeks from the date of first symptoms. Despite small numbers, Figure 2 shows us a pattern in serological positivity. In IFA the IgM positivity decreases after 8 weeks of disease, while IgG positivity peaks at 6-8 weeks. Such a pattern was not clear for ELISA mainly due to the low sensitivity of IgG.

Comparison of IFA and ELISA
IgM testing showed good similarity (86% concordance) in ELISA and IFA. For IgG concordance was 67%, mainly due to the difference in sensitivity. The ROC curves demonstrate that ELISA IgG poorly discriminated CSD patients from the control group, with an AUC of 0.67 (Figure 3).

Figure 2.
Seropositivity in (in-house) IFA (A) and ELISA (B) in relation to duration of disease. Shown is percentage of positive sera (total = 44) by IFA and ELISA for *B. henselae* IgG and IgM in relation to duration of disease in weeks. Number of sera are respectively 11, 11, 7, 5, 4 and 6 for each couple of bars.
The IFA IgM and IgG were more accurate (AUC respectively 0.73, 0.79) and the ELISA IgM performs best with an AUC of 0.85. At the point where specificity is around 92%, corresponding to the cut-offs used in this study, the curves of these last three tests are nearly superimposed. In order to compare the sensitivity of the IFA and ELISA, the cut-off of ELISA was changed to meet the same specificity as the IFA test. The specificity of IgM IFA (cut-off 1:8) was 92.9%, which was also met in the ELISA after shifting the ELISA cut-off to 4.89 SD. At this point the sensitivity of IgM ELISA (49%) was not significantly different from IFA (53%). For the IgG tests, equal specificity (82%) was met at a cut off of 1:64 (IFA) and 1.78 SD (ELISA). Using this cut-off, IgG sensitivity was significantly higher in the IFA IgG test (82.1%) than in the ELISA IgG test (29.4%) (p = 0.003). The ROC curve illustrates that the commercial IFA IgM test did not discriminate well, with an AUC of 0.53.

**B. henselae genotyping**

All lymph node or pus specimens of the patients in the CSD group were retested with a PCR that allows for amplification of a genotype-specific 185-bp 16S rRNA fragment (n = 51). Four samples were not included in this analysis because no type I or II was detectable (n = 3; amount of DNA material too low), or both genotypes I and II were identified (n = 1; possibly co-infection). Genotype I was identified in 32 (63%) lymph node samples and genotype II in 15 (29%). Table 3 shows that the sensitivity of the serological tests is higher for type I than for type II *B. henselae*, but this was only statistically significant for IgG by ELISA (p = 0.028).
Evaluation of *B. henselae* IFA and ELISA

**DISCUSSION**

In this study we evaluated the diagnostic performance of *B. henselae*-specific IgM and IgG by IFA and ELISA. We used a group of patients with proven CSD (clinical diagnosis confirmed by PCR) and a control group of patients with similar symptoms but with other confirmed diagnoses and negative PCR for *B. henselae*. In our in-house assays, IgM testing showed a low sensitivity (IFA 53% and ELISA 65%) with a specificity of 93% and 91% respectively. IgG testing was more sensitive in IFA (67%) than in ELISA (28%), with a specificity of 82% and 91%.

We included two cases without lymphadenopathy in the CSD group. One patient presented with multifocal osteomyelitis as described in a recent case report by de Kort et al. [19]. The other patient had *B. henselae* endocarditis, which usually presents as fever of unknown origin without lymphadenopathy in immunocompromised patients or in patients with a pre-existing cardiac defect. Although one could discuss whether *B. henselae* endocarditis should be considered as atypical CSD, we believe that including this patient did not flaw our results.

Compared to other reports we found relatively low specificities due to high seroprevalence in our control group. This could be explained in several ways. The controls, which all were diagnosed with diseases other than CSD, still could have had a concurrent *B. henselae* infection. Rolain et al. described mycobacteriosis in 4.2% and neoplasm in 1.2% of proven CSD patients (n = 245) [20]. The 16S rRNA PCR might have been negative in these patients as the sensitivity of this PCR is 78-100% according to previous studies [3,10,12]. Also late sample taking or small amounts of DNA available for PCR might have lead to negative PCR results.

The positive *Bartonella* IgG in the control group (seroprevalence 9-18%) may reflect past exposure to *B. henselae* as IgG remains positive long after infection [3,5]. Although serological cross-reactivity with *B. henselae* only has been reported for *Bartonella quintana* and *Coxiella burnetii* [21], cross-reactivity with other microorganisms is not unlikely [4,8]. In clinical practice, tests for diagnosing CSD need high specificity, as potential lethal disease such as

<table>
<thead>
<tr>
<th>Serological test</th>
<th>Genotype I (n=32), positive sera (%)</th>
<th>Genotype II (n=15), positive sera (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA IgM</td>
<td>66%</td>
<td>40%</td>
<td>0.10</td>
</tr>
<tr>
<td>IFA IgG</td>
<td>69%</td>
<td>67%</td>
<td>0.89</td>
</tr>
<tr>
<td>ELISA IgM</td>
<td>75%</td>
<td>53%</td>
<td>0.14</td>
</tr>
<tr>
<td>ELISA IgG</td>
<td>38%</td>
<td>7%</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

*a Chi-square Test; statistical significance is indicated by *.

**Table 3.** Sensitivity of serology in relation to *B. henselae* genotypes.
malignancies and tuberculosis can be missed in cases where CSD is falsely diagnosed [7]. Given the high seroprevalence and low sensitivity of testing for anti-\textit{B. henselae} IgG (especially in ELISA), we consider IgG testing of no additional value in diagnosing acute CSD. We analysed the effect of the duration of disease on the sensitivity (Figure 2). Despite the low numbers, we confirmed that IgM antibodies are present predominantly in the early phase of disease, while IgG antibody titers increase later [3,5,22,23]. IgM sensitivity was optimal within 6 weeks after onset of symptoms. Earlier reports describe variable patterns of antibody kinetics, often with unknown duration of the disease at sample taking [3,5,22,23]. Metzkor-Cotter et al. analysed ELISA antibody kinetics in 98 patients who suffered CSD since 1-52 weeks, with a mean serological follow-up of 35 (range 2-211) weeks [5]. They report that IgM seropositivity disappears within 3 months in 96% of the patients, while IgG titers may remain positive for > 2 years after disease onset. The present study suggests that serological confirmation of CSD is best performed in the first 6 to 8 weeks after onset of disease. In patients with suspected CSD and negative serology, \textit{B. henselae} PCR in relevant material should be used, because of the low sensitivity of serological testing [3,11].

Comparing our results to earlier reports in the literature is complicated by different study populations and definitions of CSD. Various materials and techniques have been evaluated, like different commercial versus in-house IFA or ELISA’s, detection of IgM or IgG and the use of different cut-offs [3-7,10]. Bergmans et al. tested in-house IFA and ELISA in 22 Dutch CSD patients (as defined by 2 or more classical criteria) with a control group of 60 healthy blood donors [3]. They reported sensitivities for IgM of 50% (IFA) and 71% (ELISA), and even 81% (ELISA) in PCR positive patients. Similar to our study, they found a low sensitivity for IgG (9.5% ELISA, 41% for IFA). Giladi et al. reported on the use of an in-house ELISA in Israel [6]. They compared 84 CSD patients (confirmed by PCR and/or skin test) with a similar control group (\(n = 34\)) as in the present study, but not confirmed by negative PCR. At the same cut-offs as in the present study, they found sensitivities for IgM and IgG of 48% and 77% and specificity of 100% and 94% respectively.

Comparison of various serological tests from different laboratories is complicated by the lack of standardisation. We therefore compared our in-house IgM IFA to a commercially available IgM IFA test of Focus Technologies (formerly MRL Diagnostic). The Focus product information brochure (2003) describes a specificity of 100% (tested in 75 U.S. blood donors) and a sensitivity of 41.5% in 94 CSD patients with lymphadenopathy and cat exposure. In our hands this test reached a sensitivity of only 6%. One earlier report on this commercial (agar derived) test was found in the literature. Maurin et al. compared Focus IgM and IgG IFA to an in-house IFA [24]. In a group of 68 French CSD patients (with cat contact and positive PCR) they also report low sensitivity of 1.5% (1/68) of the commercial IgM test, with a specificity of 100% in healthy blood donors (\(n = 40\)). Their in-house IgM IFA performed similarly with a sensitivity of 3% (2/68) at an cut-off titer \(\geq 1:20\). The IgG IFA sensitivity was higher in the commercial test (91%) than in the in-house assay (53%, \(p < 0.001\)) with a specificity of 100% in healthy blood donors and of 70% in patients with tuberculosis (\(n = 10\)).
Reports on other commercial tests in the literature illustrate the variety in performance of *B. henselae* serological tests. Zbinden et al. reported on 4 commercial IFA’s for IgG detection and one in-house assay [25]. Two commercial IFA tests with agar derived *B. henselae* (MRL Diagnostics and Virion) showed IgG positivity in 45-52% of healthy controls (n = 58), while two commercial IFA’s (Bion and MRL) with cell associated *B. henselae* were positive in 3.4-5.2%. Sensitivity was only tested for the MRL Vero-cell associated IgG tests being 84.6% in 22 CSD patients. Sander et al. evaluated two IFA tests, one based on larynx carcinoma cells infected with *B. henselae* (type Houston-1) and one based on Vero-cells infected with *B. henselae* and *B. quintana* (MRL Diagnostics) [7]. In a group of 42 CSD patients (20 PCR positive) and 100-270 healthy controls, IgM testing was 88-95% sensitive and 64-86% specific. IgG detection showed a sensitivity of 93-100% and a specificity of 70-73%. They measured a seroprevalence of antibodies to *B. henselae* of 30% in healthy German individuals, independent of cat-ownership.

Whether the poor performance of the commercial IgM test in our hands as well as in the French study was due to a failure in slide preparation or to the characteristics of the study population remains unclear [24]. Another explanation might be the influence of different genotypes on the sensitivity, as shown in this study. Although the number of patients was too low to achieve statistical significance except for the IgG ELISA, infection with a genotype I strain seems to be more easily detected than infections with type II strains by the current serological techniques. Possibly an infection with genotype I triggers a higher and longer lasting antibody response than genotype II and is therefore detected more easily. However, an even more plausible explanation is the single use of the homologous ATCC 49882 (genotype I) *B. henselae* strain in the IFA and ELISA, which might give a lower or negative serology result in patients infected by genotype II [13]. Since a quarter of Dutch CSD patients is not infected with *B. henselae* type I, the use of locally isolated 16S type I and type II *B. henselae* strains in serologic assays may improve performance [16].

In conclusion, improving serology for diagnosing CSD remains a challenge as no ideal serological test has been described yet. In our population the detection of *B. henselae* IgM antibodies by IFA or ELISA in patients suspected of CSD is highly confirmative for the diagnosis with a positive predictive value of 87%. IgG testing is of limited use, as sensitivity and specificity will be low in populations with high seroprevalence. Given the low sensitivity of the serologic assays, *B. henselae* PCR should be considered in patients with suspected CSD and negative serology. As the sensitivity of serological testing seems dependent on the *B. henselae* genotype causing the infection, addition of several locally isolated strains might improve the quality of the tests.

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