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

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ABSTRACT

Cat-scratch disease (CSD), caused by *Bartonella henselae* infection, can mimic malignancy and can manifest atypically. Reliable serological testing is therefore of great clinical importance. 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 (patients with similar symptoms, but who were *B. henselae* PCR-negative and had an alternative confirmed diagnosis) were tested for anti-*B. henselae* IgM and IgG by IFA and ELISA. A commercially available IFA test for IgM had a sensitivity of 6%. In-house assays for IgM showed specificities of 93% (IFA) and 91% (ELISA), but 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 for genotype I (38–75%) than for genotype II (7–67%) infections, but this was only statistically significant for IgG ELISA ($p < 0.05$). In conclusion, detection of IgM against *B. henselae* by in-house ELISA and IFA was highly specific 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 investigation, including PCR. Adding locally isolated (e.g., genotype II) *B. henselae* strains to future tests might improve the sensitivity.

Keywords *Bartonella henselae*, cat-scratch disease, diagnosis, ELISA, immunofluorescence assay, PCR

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INTRODUCTION

Bartonella 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 hepatis. 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 for which antibiotic treatment needs to be considered.

For over 30 years, diagnosis of CSD has relied on clinical criteria and skin tests [1], but the simplicity of serology means that this approach is now usually the first step in the confirmation of suspected CSD [3,4]. Indirect immunofluorescence assay (IFA) and ELISA are used for detection of anti-*B. henselae* antibodies in serum. Although IFA is the technique used most widely, IFA is more time-consuming than ELISA, and interpretation might be less objective [5]. Previous evaluations of serological tests reported a range of sensitivities and specificities, depending on the study population, definitions of CSD, and the materials and techniques used [3,6,7]. *B. henselae* is difficult to culture from patients, but PCR is

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highly specific and sensitive for detecting *B. henselae* DNA in pus or lymph node specimens. The sensitivity of PCR is dependent on the target genes and the particular patient group [3,8–12]. The main disadvantage of PCR is the need to obtain invasive samples of pus or other relevant tissue [10]. Although recent studies have suggested a more complex classification of *B. henselae*, two *B. henselae* 16S rRNA genotypes that are associated with cases of CSD have been identified previously [13–15]. Genotypes I (corresponding to serotype Houston-1) and II (serotype Marseille) can be distinguished by PCR [16]. After the discovery of genotype II, Drancourt *et al*. [13] suggested that the precise genotype involved might influence the accuracy of serological tests, although this has not been confirmed.

In the present study, the diagnostic performance of IFA and ELISA was evaluated in patients with a clinical presentation consistent with CSD, confirmed by PCR, and in a clinically relevant control group with negative PCR results. A commercially available IFA was compared with in-house assays, and the influence of the two different *B. henselae* genotypes on the sensitivity of these serological assays was examined.

MATERIALS AND METHODS

Patient population

Material from patients in The Netherlands with suspected *B. henselae* infection is sent to two national reference laboratories, the National Institute of Public Health and the Environment, Bilthoven (RIVM) and the Regional Public Health Laboratory, Tilburg (RPHL). Patients with material sent for both *B. henselae* PCR and serology, with sufficient serum stored for additional testing, were selected from the laboratory databases of both centres. The referring physicians were asked to complete an anonymised form that provided clinical and epidemiological data (age, gender, symptoms of disease, duration of illness, cat contact and final diagnosis). The patients were divided into the study group (CSD group) and the negative control group according to clinical data and PCR results (Fig. 1).

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

Diagnosis	Number of patients (%)
Infection	20 (36%)
Mycobacterial (atypical)	8 (40%)
Mycobacterial (typical)	4 (20%)
Bacterial, other	7 (35%)
Viral	1 (5%)
Malignancy	21 (38%)
Hodgkin's lymphoma	6 (30%)
Non-Hodgkin's lymphoma	5 (25%)
Other	10 (50%)
Immunological disorder[a]	8 (14%)
Congenital cyst/fistula	5 (8.9%)
Other diagnosis	2 (3.6%)

[a]Reactive lymphadenitis in human immunodeficiency virus infection, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, autoimmune lymphoproliferative syndrome and Devic's disease.

CSD group. The CSD group included patients with a clinical presentation of CSD, based on retrospective analysis of clinical data and a PCR test positive for *B. henselae*. Clinical presentation of CSD was defined as lymphadenitis or an atypical presentation of *B. henselae* infection in the absence of another diagnosis. The combination of matching clinical data and a positive PCR result was considered to be the reference standard for a proven infection with *B. henselae*.

Control group. The control group included patients whose material was sent for *B. henselae* testing, but who eventually had a different clinical diagnosis (Table 1) and a PCR test negative for *B. henselae*.

Exclusions. Exclusions included patients who did not meet the criteria for the above two groups, or for whom insufficient clinical data concerning their diagnoses were available.

Laboratory techniques

All serum samples were analysed for *B. henselae*-specific IgM and IgG antibodies by ELISA at RIVM, and by IFA at 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 −20°C.

IFA. In-house antigen slides for detection of IgM and IgG antibodies to *B. henselae* were prepared as described previously [3]. A bacterial suspension of *c.* 10^8 CFU/mL was made from *B. henselae* ATCC 49882 (*B. henselae* type Houston-1), grown on Columbia agar supplemented with sheep blood 5% v/v. The suspension was mixed with egg yolk emulsion

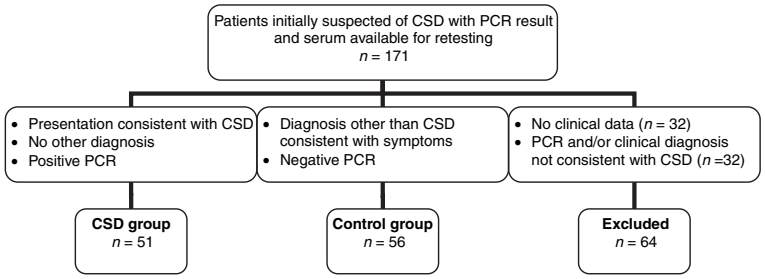

Fig. 1. Subdivision of patients initially suspected of having cat-scratch disease (CSD) on the basis of clinical analysis and PCR tests for *Bartonella henselae*, resulting in three groups: CSD group, control group and excluded patients.