Evaluation of Indirect Fluorescence Antibody Assay for Detection of 
Bartonella clarridgeiae and Seroprevalence of B. clarridgeiae among 
Patients with Suspected Cat Scratch Disease

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The possibility of Bartonella clarridgeiae being a causative agent of cat scratch disease (CSD) was investigated 
by using indirect fluorescence antibody assays with 288 suspected CSD patients. Immunoglobulin G antibody 
to noncocultivated B. clarridgeiae was suitable only for detection of B. clarridgeiae antibody. Significant 
cross-reactivity between Bartonella henselae and B. clarridgeiae was noted, and no CSD case caused by B. 
clarridgeiae was detected.

The predominant causative pathogen for cat scratch disease 
(CSD) is Bartonella henselae (1, 2, 6). Since the isolation of 
Bartonella species from CSD patients is difficult, indirect fluo-
rescence antibody assays (IFA) for detection of antibodies to 
Bartonella species is commonly used for serological diagnosis 
and epidemiological studies (5, 14, 19, 23).

Recently, B. clarridgeiae was suggested as an additional caus-
ative agent of CSD (9, 11). This prompted us to reanalyze sera 
of patients clinically suspected of having CSD, and we inves-
tigated the prevalence of immunoglobulin G (IgG) and IgM 
antibodies to B. clarridgeiae among such patients in Japan.

For detection of IgG and IgM antibodies to B. clarridgeiae, 
two IFA protocols were evaluated. To investigate cross-reac-
tivity between B. henselae and B. clarridgeiae, two IFA protocols were evaluated. To investigate cross-reac-
tivity between B. henselae and B. clarridgeiae, 1 ml of each 
sample from 20 patients with serum titers of IgG antibody to B. 
clarridgeiae of ≥1:128 was absorbed with 2 mg of B. henselae 
ATCC 49982 or B. clarridgeiae ATCC 51734 and then tested by 
IFA. For scanning electron microscopy, B. clarridgeiae cocul-
tivated with Vero cells on small pieces of cover glass was 
incubated with a 1/50 dilution of the tested sera for 90 min at
35°C in 5% CO₂, followed by the protein A-gold labeling (21),
and processed and examined as described previously (20). So-
dium dodecyl sulfate-polyacrylamide gel electrophoresis and 
Western blot analysis were performed as described previously
(10, 22).

Comparison of two IFA protocols. (i) IgG and IgM antibod-
ies to cocultivated B. clarridgeiae. IgG antibody to B. clarridge-
iae was analyzed by IFA using B. clarridgeiae cocultivated with 
Vero cells for 24 and 96 h at 35°C in 5% CO₂, as described 
previously (19). B. clarridgeiae cocultivated with Vero cells for
24 h strongly reacted with 20 healthy human sera (1:2,048 to 
1:4,056) (Fig. 1A). When B. clarridgeiae was cocultivated for
96 h, the titers increased by about eight times (1:16,384 to
1:32,768) compared to those for cocultivation for 24 h. These
results suggested that titers of IgG antibody to cocultivated B. 
clarridgeiae might depend on cocultivation time, indicating its 
nonspecific reaction. Alternatively, cocultivation with Vero 
cells might enhance the expression of some surface antigenic

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FIG. 1. IFA using B. clarridgeiae with healthy human or CSD pa-

tient's serum (1:128 dilution). Cocultivated bacteria with Vero cells 
for 24 h strongly reacted with healthy human serum (A), whereas bacteria 
cocultivated for 0.5 h did not react (B) but strongly reacted with the 
CSD patient's serum (C).
determinants in *B. clarridgeiae*. Scanning electron microscopy also showed its nonspecific reaction (Fig. 2). Numerous gold particles were labeled on the surface of bacteria added to Vero cells and cocultivated with Vero cells for 72 h (A), but only a few gold particles were observed on the surface of bacteria added to Vero cells and incubated for 3 h (B). Bar, 100 nm.

FIG. 2. Immuno-gold labeling of *B. clarridgeiae* with healthy human serum. The healthy human serum nonspecifically reacted with bacteria cocultivated with Vero cells for 72 h (A), but only a few gold particles were observed on the surface of bacteria added to Vero cells and incubated for 3 h (B). Bar, 100 nm.

(ii) IgG and IgM antibodies to noncocultivated *B. clarridgeiae*. Of the 20 healthy individuals, 1 had a titer of IgG antibody of 1:64 for *B. clarridgeiae* grown on the rabbit blood agar medium for 7 days at 35°C in 5% CO₂. The other 19 had titers that were <1:64. Nonspecific reaction was not observed in any of the 20 individuals. IgM antibody to *B. clarridgeiae*, analyzed for 100 healthy controls, was positive in 20 (20%), with titers of 1:20 to 1:160. Thus, IgM for *B. clarridgeiae* was unsuitable because of false-positive results with healthy controls.

 Altogether, IgG antibody to noncocultivated *B. clarridgeiae* was suitable only for detection of *B. clarridgeiae* antibody.

**The association of *B. clarridgeiae* with CSD.** Of the total of 100 sera from healthy individuals with no past history of either lymph node swelling or cat scratch or bite, 3 (3.2%) of 94 sera serologically negative for IgG antibody to *B. henselae* and 3 (50.0%) of 6 sera with titers of IgG antibody to *B. henselae* of 1:64 to 1:128 were positive for IgG antibody to *B. clarridgeiae*, with titers of 1:64 to 1:128 (Table 1).

Sera from 288 patients (221 children and 67 adults) clinically suspected of having CSD because of either lymphadenopathy or fever of unknown origin and a previous history of cat scratch or contact were reevaluated for IgG antibody to *B. clarridgeiae*. Paired acute-phase and convalescent-phase sera were obtained from 35 patients. They were divided into three groups by titers of IgG antibody to *B. henselae*. Group 1 included 145 samples with titers of <1:64, group 2 included 59 patients whose sera had titers of 1:64 to 1:128, and group 3 included 84 samples with titers of ≥1:256. In group 1, 4 (2.8%) were positive for *B. clarridgeiae* with a titer of 1:64, and 14 (23.7%) of group 2 and 45 (53.6%) of group 3 were positive for IgG antibody to *B. clarridgeiae* with titers of 1:64 or more (Table 1). One patient’s serum had high titers of 1:1,024 for IgG antibody to *B. clarridgeiae*. Thus, the positive rate for *B. clarridgeiae* IgG increased significantly in proportion to the increase of the titer of *B. henselae* IgG among suspected CSD patients (*P* < 0.0001 for group 1 versus group 2; *P* = 0.02 for group 2 versus group 3). None of paired sera from 35 patients showed a fourfold rise in titers of IgG antibody to *B. clarridgeiae*. There was no difference in the positive rate for IgG for *B. clarridgeiae* between the control group and group 1 (*P* = 1.000).

**Serological cross-reaction.** When the sera were absorbed with *B. henselae*, titers of IgG for both *B. henselae* and *B. clarridgeiae* were significantly reduced or disappeared (Table 2). When the sera were absorbed with *B. clarridgeiae*, titers of IgG antibody to *B. henselae* did not change, whereas titers of

**TABLE 1. Titers of IgG antibodies to *B. henselae* and *B. clarridgeiae* in sera of 100 healthy controls and 288 patients with suspected CSD**

<table>
<thead>
<tr>
<th>Group</th>
<th>Titer of IgG antibody to <em>B. henselae</em></th>
<th>No. of samples tested</th>
<th>No. of sera with titer of IgG antibody to <em>B. clarridgeiae</em> of:</th>
<th>No. (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>&lt;64</td>
<td>94</td>
<td>91 2 1 0 0 0</td>
<td>3 (3.2)</td>
</tr>
<tr>
<td></td>
<td>64–128</td>
<td></td>
<td>6 3 2 1 0 0</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td>Patients</td>
<td>&lt;64</td>
<td>145</td>
<td>141 4 0 0 0</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td></td>
<td>64–128</td>
<td>59</td>
<td>45 9 5 0 0</td>
<td>14 (23.7)</td>
</tr>
<tr>
<td></td>
<td>≥256</td>
<td>84</td>
<td>39 30 12 2 0</td>
<td>45 (53.6)</td>
</tr>
</tbody>
</table>

* a *B. henselae* cocultivated with Vero cells.

* b *B. clarridgeiae* not cocultivated with Vero cells.

* c Titer of IgG antibody to *B. henselae* was 1:4,096.
IgG antibody to *B. clarridgeiae* were not detected. Western blotting with a CSD patient’s serum showed that it reacted predominantly with a 58-kDa protein of *B. henselae* and 58- and 37-kDa proteins of *B. clarridgeiae* (Fig. 3B). The serum absorbed with *B. henselae* resulted in diminished reaction, and there was no band reacting with either strain (Fig. 3C). On the contrary, the serum absorbed with *B. clarridgeiae* still reacted against a 73-kDa protein of *B. henselae* (Fig. 3D). Although this protein band was thin, its intensity was the same as that of unabsorbed serum (Fig. 3B and D).

In view of the facts that cats are infected with both *B. henselae* and *B. clarridgeiae* simultaneously (4, 8, 12), *B. clarridgeiae* could be a causative agent of CSD (9, 11), and there was cross-reaction between *B. henselae* and *B. clarridgeiae*, as

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**TABLE 2.** Titers of IgG antibodies to both *B. henselae* and *B. clarridgeiae* in sera of patients with CSD before and after absorption with *B. henselae* or *B. clarridgeiae*

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Titer of IgG Before absorption</th>
<th>Titer of IgG After absorption with <em>B. henselae</em></th>
<th>Titer of IgG After absorption with <em>B. clarridgeiae</em></th>
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<tbody>
<tr>
<td></td>
<td><em>B. henselae</em></td>
<td><em>B. clarridgeiae</em></td>
<td><em>B. henselae</em></td>
</tr>
<tr>
<td>1</td>
<td>128</td>
<td>128</td>
<td>&lt;64</td>
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<tr>
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</tr>
<tr>
<td>20</td>
<td>4,096</td>
<td>1,024</td>
<td>1,024</td>
</tr>
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**FIG. 3.** Cross-reaction between *B. henselae* and *B. clarridgeiae* in CSD patient's serum. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell protein of *B. henselae* (lane 1) and *B. clarridgeiae* (lane 2) incubated on blood agar. (B to D) Immunoblot analyses by using the serum from a CSD patient (B), the serum absorbed with *B. henselae* cells (C), and the serum absorbed with *B. clarridgeiae* cells (D). Arrowheads show that the 73-kDa band still reacted with the serum after absorption with *B. clarridgeiae* cells.
shown between *B. henselae* and *Bartonella quintana* (5, 7, 13, 15, 17, 18), serological diagnosis of CSD should be carried out with caution (3), or a specific marker, such as *B. clarridgeiae* flagellin (16) as antigen, is recommended to overcome the cross-reaction. With respect to association of *B. clarridgeiae* with CSD, our study failed to demonstrate a case of CSD caused by *B. clarridgeiae*. Further accumulation of CSD patients and improvement of the serological method would shed more light on the role of *B. clarridgeiae* in the pathogenesis of CSD.

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REFERENCES