

Prevalence of *Bartonella henselae* in granulomatous lymphadenitis : A useful tool for the diagnosis of cat-scratch disease by polymerase chain reaction.

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Bartonella Henselae is the causative agent of human cat-scratch disease (CSD). CSD can be diagnosed by histopathological examination, and by the enzyme immunoabsorbant assay (EIA) and polymerase chain reaction (PCR) methods, which detect *B. Henselae* DNA. Histopathological findings of CSD include granulomatous lymphadenitis with or without abscess formation. We examined 36 specimens of granulomatous lymphadenitis, including 15 abscess-forming granulomatous lymphadenitis, 9 non-abscess-forming granulomatous lymphadenitis, 10 tuberculosis and 2 sarcoidosis by PCR with primers specific for *B. Henselae* and *Bartonella quintana*. As controls, we examined 79 cases of non-granulomatous lymphadenitis, including 7 with dermatopathic lymphadenopathy, 18 Kikuchi's lymphadenitis (histiocytic necrotizing lymphadenitis), 18 follicular hyperplasia, 18 paracortical hyperplasia and 18 non-specific lymphadenitis. The PCR method identified *B. henselae* in 10 of 15 (67%) patients with abscess-forming granulomatous lymphadenitis and 2 of 9 (22%) with non-abscess-forming granulomatous lymphadenitis, but none in tuberculosis, sarcoidosis and non-granulomatous lymphadenitis. *B. henselae* was persistently detected in all but one patient from the appearance of symptoms to 4 months from onset in patients with abscess-forming and non-abscess-forming granulomatous lymphadenitis. Our results suggest that the PCR method is useful for establishing the diagnosis of CSD by detecting *B. henselae*.

Key words lymphadenitis, abscess-forming lymphadenitis, PCR

INTRODUCTION

Bartonella henselae (*B. henselae*) is a small, pleomorphic, gram-negative bacillus and the causative agent of human cat scratch disease (CSD)¹⁻⁷ as well as several serious complications of infections, including bacillary angiomatosis and bacillary peliosis^{8,9}. The diagnosis of CSD is based on a history of exposure to cats with recent scratch wounds or papules, tender regional lymphadenopathy, a positive skin test, and negative routine bacterial cultures. CSD can also be diagnosed by serological examination for *B. henselae*, such as an enzyme immunoabsorbant assay (EIA)^{1,10,11} or a genetic assay with polymer-

ase chain reaction (PCR) using primers specific for *B. henselae*^{3,5,7,12-14}.

CSD presents with the characteristic features of granulomatous lymphadenitis with or without abscess formation^{15,16}. Abscess-forming granulomatous lymphadenitis (suppurative granulomatous lymphadenitis) is a form of reactive lymph-node lesion that occurs during the course of various infections, such as CSD, tular-emia, lymphogranuloma venereum, *Yersinia* lymphadenitis, and mycobacterial infection that are mostly caused by atypical mycobacteria, and fungal infection¹⁶. Therefore, the histopathological findings in CSD are not diagnostic and may be mistaken for other diseases that present as granulomatous lymphadenitis.

There are reports indicating that Warthin-Starry silver staining is very useful to make a diagnosis of abscess-forming granulomatous

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lymphadenitis, especially CSD¹⁷⁻²⁰. However, in practice, Warthin-Starry silver staining is difficult to perform and interpretation of the results is often subjective. Furthermore, it is usually difficult to identify the bacilli in lymph nodes stained by this method, even if the patients present with the clinical features typical of CSD. We need better investigational tools to detect this bacillus. In this study, the PCR method was applied to patients with granulomatous lymphadenitis to determine if this method was useful for diagnosis of CSD.

Materials and Methods

Patients and specimens

We selected lymph-node specimens from 36 patients with granulomatous lymphadenitis that were filed in the Department of Pathology, Fukuoka University. They included 15 samples with abscess-forming granulomatous lymphadenitis (6 males and 9 females, mean age 45.9 years, range 11-74), 9 with non-abscess-forming granulomatous lymphadenitis (4 males and 5 females, mean age 26.5 years, range 1-49), 10 with tuberculosis (3 males and 7 females, mean age 54.1 years, range 20-76) and 2 with sarcoidosis (2 females, mean age 64.0 years, 51 and 77). Samples of non-granulomatous lymphadenitis from 79 patients were used as controls, including 7 with dermatopathic lymphadenopathy, 18 with Kikuchi's lymphadenitis (histiocytic necrotizing lymphadenitis), 18 with follicular hyperplasia, 18 with paracortical hyperplasia and 18 with non-specific lymphadenitis.

Histopathological examination

Resected lymph nodes were fixed in buffered formalin and embedded in paraffin. Paraffin-embedded sections were stained for histopathological examination with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), and reticulin stains. Histochemistry with alpha-naphthol AS-D chloroacetate (NASDA) was performed to detect neutrophils.

Culture samples

B. henselae (ATCC strain no. 49793) and *B.*

quintana (ATCC strain no. 49927) were grown on heat-infusion agar plates supplemented with 5% defibrinated sterile rabbit blood and incubated for 3 to 5 days at 34°C in 5% carbon dioxide. *Afipia felis* (ATCC strain no. 53690), an organism that was previously thought to cause CSD²¹, was grown on charcoal-yeast extract agar plate for 2 to 3 days at 32°C without carbon dioxide. DNA was extracted from each bacterial strain and used as a positive or negative control for our PCR method.

PCR method

We obtained DNA from frozen tissue or paraffin-embedded tissue of the biopsied lymph node. Specific primers and probes were prepared in our laboratory by methods reported previously^{3,22,23} (Table 1). Amplification was carried out with a GenAmp DNA amplification reagent kit and DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT). The selected PCR condition was based on previous descriptions^{3,22,23}. For subsequent PCR using clinical samples, 10 pg (in 10 µl) of DNA extracted from either *B. henselae*, *B. quintana* or *A. felis* was used as a positive or negative control. PCR was performed through 35 cycles consisting of denaturing at 94°C for 1 min, annealing at 52°C for 2 min and extension at 70°C for 1.5 min. Ten µl from each PCR assay was electrophoresed through a 4% agarose gel, stained with ethidium bromide, and photographed. The presence of a 153-bp band was considered positive. In addition, the agarose gel was placed onto a nylon membrane and Southern blot hybridization was performed to determine the presence of *B. henselae* or *B. quintana* using specific probes labeled with ³²P. In addition, to confirm that PCR products from several clinical samples were infected with *B. henselae* the dot-blot assay was performed with specific probes for both types of bacteria.

Table 1. Primers and probes for PCR assay

Primers for <i>B. henselae</i> and <i>B. quintana</i>	
CAT-1:	5'-GATTCAATTGGTTTGAAGGAGGCT-3'
CAT-3:	5'-TTGCAATACGCTTTGCTAGATCACGG-3'
Probes for <i>B. henselae</i> and <i>B. quintana</i>	
<i>B. henselae</i> Rh1:	5'-GGTGCCTTAATTACCGATCC-3'
<i>B. quintana</i> Rq1:	5'-GGCGCTTTGATTACTGATCC-3'

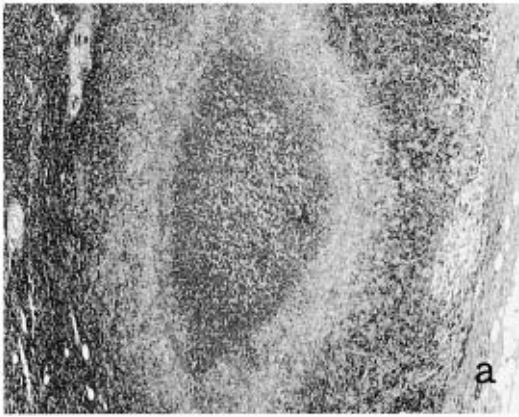


Fig. 1a. Abscess-forming granulomatous lymphadenitis. Central necrosis with severe infiltration of neutrophils is noted. (H & E stain)

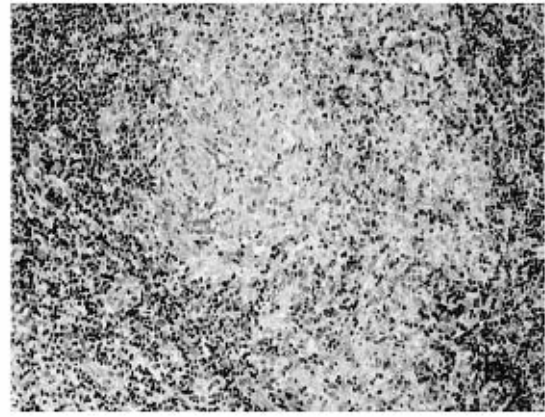


Fig. 1b. Non-abscess-forming granulomatous lymphadenitis. A granulomatous lesion without neutrophilic infiltration is present. (H & E stain)

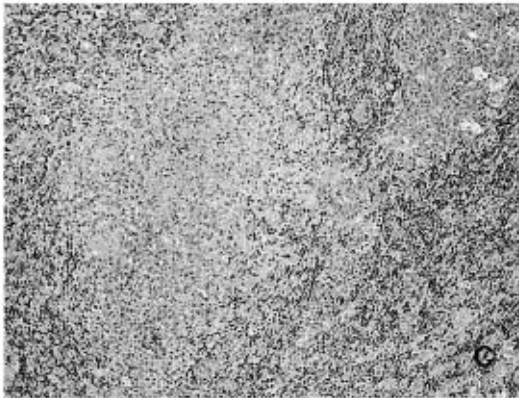


Fig. 1c. Abscess-forming granulomatous lymphadenitis. Some neutrophils are scattered. (H & E stain)

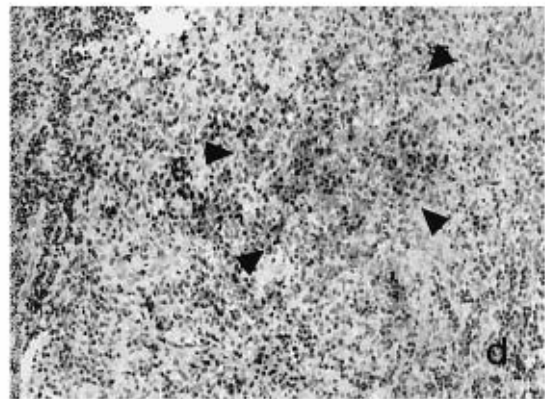


Fig. 1d. Abscess-forming granulomatous lymphadenitis. Some NASDA positive neutrophils (arrows) are present. (Histochemistry of NASDA)

Results

Fig. 1 shows typical histopathological findings in representative cases of abscess-forming granulomatous lymphadenitis and non-abscess forming granulocytic lymphadenitis. Using the PCR method, *B. henselae* was detected in 10 of 15 (67%) abscess-forming granulomatous lymphadenitis and 2 of 9 (22%) of non-abscess-forming granulomatous lymphadenitis, but in none of the cases with tuberculosis, sarcoidosis and non-granulomatous lymphadenitis, including all cases of dermatopathic lymphadenopathy, Kikuchi's lymphadenitis (histiocytic necrotizing lymphadenitis), follicular hyperplasia, paracortical hyperplasia and non-specific lymphadenitis. A summary of the results is shown in Table 2.

No clinical details were available in two with each form of abscess-forming and non-abscess-forming granulomatous lymphadenitis.

Clinical records were available to determine the period between onsets of initial symptoms and the time of biopsy in 13 patients with abscess-forming and 7 with non-abscess-forming granulomatous lymphadenitis. A summary of the results is shown in Table 3. The mean period from initial symptoms to the time of biopsy in patients with abscess-forming and non-abscess-forming granulomatous lymphadenitis was 4.6 and 8.4 weeks, respectively. In patients with abscess-forming granulomatous lymphadenitis, the interval for patients presenting with *B. henselae* (3.1 weeks) was shorter than for patients without *B. henselae*, in contrast, there was no

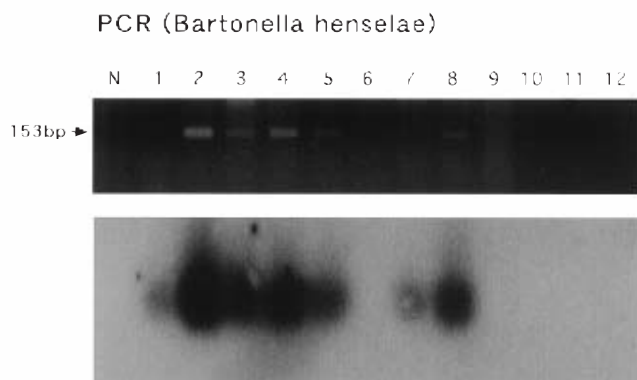


Fig. 2. PCR for *Bartonella henselae*. Ethidium bromide-stained gel of a PCR analysis using primers *B. henselae*. An amplified fragment of 153 bp is seen in lanes 1-5, 7 and 8. Southern blotting of PCR-amplified analysis using ³²P-labeled specific probes of *B. henselae* confirm infection by *B. henselae*. Lanes: N, negative control; 1-7, abscess-forming granulomatous lymphadenitis; 8, positive control (*B. henselae*, ATCC strain No. 49793); 9-12, tuberculosis. Period from initial symptoms to the time of biopsy in lanes 1 to 7 was 3, 3, 4, 3, 7, 11, and 2 weeks, respectively.

difference of time-to-biopsy with and without *B. henselae* in patients with non-abscess-forming granulomatous lymphadenitis. Our analysis indicated that *B. henselae* was found in the lymph nodes up to 4 months after the onset of symptoms in all cases of abscess-forming granulomatous lymphadenitis and all but one case of non-abscess-forming granulomatous lymphadenitis.

DISCUSSION

In most patients, CSD manifests as a self-limiting lymphadenitis after an inoculation injury. Such injury is usually inflicted by a cat scratch²⁴. Pleomorphic bacilli found in typical granulomatous lesions by the Warthin-Starry

silver stain long have been believed to cause this disease¹⁷. In 1988, English, et al. isolated a gram-negative bacillus that is now termed *A. felis* in patients with clinical features of CSD^{21,25}. Kojima and colleagues²⁶ reported that Warthin-Starry silver-stain-positive bacteria were seen in 70% of patients with abscess-forming granulomatous lymphadenitis associated with exposure to cats. However, in our experience, the detection of bacilli by Warthin-Starry silver staining is difficult. Furthermore, *B. henselae* is cumbersome to cultivate in routine culture media. Therefore, alternative diagnostic techniques are needed urgently.

One technique, EIA, was developed using anti-*B. henselae* antibodies²⁷. Several studies

Table 2. The prevalence of *Bartonelae Henselae*.

Histological diagnosis	No. cases	Positive	%
Granulomatous lymphadenitis			
Abscess-forming	15	10	67
Non-abscess-forming	9	2	22
Tuberculosis	10	0	0
Sarcoidosis	2	0	0
Non-granulomatous lesions			
Dermatopathic lymphadenitis	7	0	0
Kikuchi's lymphadenitis	18	0	0
Follicular hyperplasia	18	0	0
Paracortical hyperplasia	18	0	0
Non-specific lymphadenitis	18	0	0

Table 3. Period from initial symptoms to the time of biopsy.

	No. cases	Mean (weeks)	Range (weeks)
Abscess-forming granulomatous lymphadenitis			
<i>B. henselae</i> +	8	3.1	2-4
<i>B. henselae</i> -	5	7.0	2-11
Non-abscess-forming granulomatous lymphadenitis			
<i>B. henselae</i> +	2	4.5	2-7
<i>B. henselae</i> -	5	10.0	2-36

indicated that the majority of patients with clinically-suspected CSD carried significantly elevated antibody titers to *Rochalimaea henselae*, now termed *B. henselae*^{1,10,11}. Efforts have continued to design other diagnostic tools for the detection of *B. henselae*. In this regard, immunohistochemical studies using specific antibodies revealed that 33% of 46 patients exhibiting clinical features consistent with CSD were positive²⁸.

PCR methods have effectively detected *B. henselae* in several study^{3,5,6,23}. Mouritsen and colleagues¹² found *B. henselae* DNA in 7 (54%) of 13 lymph-node samples with morphological features suggestive of CSD, whereas none of the seven negative controls yielded positive results. *B. henselae* DNA was identified in 27 of 42 (64%) histologically-defined CSD patients and in 23 of 34 (68%) patients defined by both clinical and histological characteristics²³. The PCR analysis in our study detected *B. henselae* in 10 of 15 (67%) cases with abscess-forming granulomatous lymphadenitis, which represents the acute form of CSD. Our results are similar to those of previous studies^{23,12,13}. On the other hand, only 2 of 9 (22%) patients with non-abscess-forming granulomatous lymphadenitis, which is thought to be a late stage of CSD, were positive for *B. henselae* by the PCR method. Thus, it appears that the prevalence of *B. henselae* in patients with non-abscess-forming granulomatous lymphadenitis was lower than in those with abscess-forming granulomatous lymphadenitis.

A PCR assay designed to amplify DNA from *B. henselae* and *A. felis* was used by Bergmans, and colleagues on aspirates of suppurative lesions from 89 skin test-positive patients with CSD. *B. henselae* DNA was found in 96% of the samples⁷. The reported rate is apparently higher than in the present and previous studies. Detection of *B. henselae* DNA in such cases may correlate with the presence of abundant necrotic tissue with neutrophilic infiltration. Thus, clinical samples containing pus seem to be suitable for the detection of *B. henselae*. The difference in prevalence of *B. Henselae* detected between granulomatous lymphadenitis with abscess and without abscess is probably influenced by the stage of CSD. In addition, antimicrobial therapy should also have influenced the detection rates of *B. henselae* when clinical samples were subjected to PCR assay.

Our patients were not evaluated for their

history of exposure to cats, thus the study sample may include non-CSD cases. In some stages of CSD, no neutrophils, only debris and macrophages, were present. In such cases, CSD may be misdiagnosed as Kikuchi's lymphadenitis or another disease exhibiting the features of granulomatous lymphadenitis. So an accurate diagnosis can be achieved only by demonstrating the presence of *B. henselae* by the PCR method. This method is quite specific and diseases other than CSD were all negative for *B. henselae*.

Based on these results, we conclude that the PCR method, using specific primers and probes, is useful as a diagnostic tool to detect *B. henselae* in cases of suspected CSD.

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