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Cat Scratch Disease: the Rare Role of *Afipia felis*

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Since its isolation in 1988, *Afipia felis* has been associated with cat scratch disease (CSD) in only one report and its role in CSD has been questioned. We have cultured *A. felis* from a lymph node of a patient with CSD. 16S rRNA gene sequencing, DNA relatedness studies, fatty acid analysis, and PCR of the *A. felis* ferredoxin gene showed that the isolate is identical to the previously reported *A. felis* isolate. To determine the role of *A. felis* in CSD, PCR of the 16S rRNA gene followed by hybridizations with specific probes were performed with lymph node specimens from CSD patients. All 32 specimens tested positive for *Bartonella henselae* and negative for *A. felis*. We conclude that *A. felis* is a rare cause of CSD. Diagnostic tests not conducive to the identification of *A. felis* might cause the diagnosis of CSD due to *A. felis* to be missed.

Since its first description in 1950 (11) cat scratch disease (CSD) was suspected to be an infectious process. In 1988, English et al. (14) successfully cultured the Cat Scratch Disease bacillus, later designated *Afipia felis*, from the lymph nodes of 10 patients with CSD. Three additional isolates of *A. felis*, cultured from the lymph nodes of two pediatric patients at one medical center, were described by Brenner et al. (9) in 1991. Since these publications, there have been no reports of the isolation of *A. felis* from CSD patients; however, numerous reports from various countries have identified *Bartonella henselae* (formerly *Rochalimaea henselae*) as the major etiologic cause of CSD. *B. henselae* has been cultured from the lymph nodes and pus of patients with CSD as well as from cat blood, anti-*B. henselae* immunoglobulin M and immunoglobulin G antibodies have been detected in the serum of CSD patients, and *B. henselae* DNA has been identified by PCR in the lymph nodes of patients with clinical CSD and in pus used for the preparation of the CSD skin test (3, 4, 7, 12, 18, 25, 28). As a result, recent studies have focused their diagnostic efforts on the identification of *B. henselae*. In this report, a patient with CSD from whom *A. felis* was cultured and characterized is described.

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MATERIALS AND METHODS

Bacterial strains. *A. felis* ATCC 53690^T was provided by D. J. Wear (Armed Forces Institute of Pathology, Washington, D.C.). The *Afipia* strains *A. clevelandensis* ATCC 49720^T, *A. broomeae* ATCC 49717^T, and *Afipia* genomospecies 1 to 3 (strains ATCC 49721, ATCC 49722, and ATCC 49723, respectively), whose DNAs were used for the purpose of comparison with *A. felis*, have been described previously (9). *B. henselae* 87-66^T (ATCC 49793^T) was described by Welch et al. (27). BhTA-2 and BhTA-3 are *B. henselae* isolates cultured at Tel Aviv Medical Center from patients with CSD and have been described previously (6). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are clinical isolates from the microbiology laboratory at the Tel Aviv Medical Center. *Borrelia burgdorferi* B31 was obtained from M. L. Lovett (University of California at Los Angeles School of Medicine, Los Angeles, Calif.).

Skin test. Pus was aspirated from the lymph nodes of patients with CSD and was prepared as described previously (6). A positive CSD skin test was defined by induration of ≥5 mm at 48 to 72 h.

Extraction of DNA and PCR amplification. DNA was extracted from bacteria and clinical samples as described previously (6). Primers corresponding to the ferredoxin and the 16S rRNA genes were synthesized (Tal Ron Scientific Products Ltd., Rehovot, Israel) according to described for previously published sequences (Table 1) and were used for PCR amplification as described previously (7, 26).

Dot blot hybridization assay. The 16S rRNA PCR products were spotted, in duplicate, onto two identical membranes and were hybridized with either *A. felis*- or *B. henselae*-specific probes (Table 1). Labelling of the probes and hybridization assay were performed as described previously (5). Tetramethylammonium chloride (Sigma Chemical Co., St. Louis, Mo.) was used in the posthybridization washes to eliminate disparities in the melting temperatures for the oligonucleotide probes (17), thus enabling the hybridization of *B. henselae*- and *A. felis*-specific probes under identical conditions.

Cellular fatty acid analysis. Whole-cell fatty acid analysis was performed with cultures harvested from plates containing heart infusion agar supplemented with 5% rabbit blood and incubated at 30°C for 5 days. The preparation and analysis of fatty acid methyl esters were done by the techniques developed by Miller and Berger (19).

Sequencing of the 16S rRNA gene. The 16S rRNA gene from strain AfTA-1 was amplified and sequenced as described previously (10). Sequence analysis for AfTA-1 and *A. felis* ATCC 53690^T was performed with the Phylogeny Inference Package (Phylip), version 3.51c (14a).

DNA relatedness. The cultivation of bacteria, purification and radiolabelling of DNA, and the hydroxyapatite method used for DNA hybridization have been described previously (8). Hybridization reactions were performed at 65°C. Levels of divergence were calculated to the nearest 0.5%, as described previously (8).

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain AfTA-1 was deposited in the GenBank database under accession no. AF003937.

RESULTS

Case history. A 21-year-old white female presented to the otorhinolaryngology service with right supraclavicular lymphadenopathy of 3 weeks' duration. She was afebrile, felt well, and had no other complaints. The patient owned a kitten which she hugged and kissed and was often scratched during play. Physical examination was unremarkable except for bilateral

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TABLE 1. Primers and probes used for PCR and hybridization assays

Oligonucleotide name	Oligonucleotide sequence (5′→3′)	Target organism	Target gene	Nucleotide position	Product size (bp)	Reference
p93E	CCGCACAAGCGGTGGAGCA	Eubacteria	16S rRNA	931–949[a]	480	26
p13B	AGGCCCGGGAACGTATTCAC	Eubacteria	16S rRNA	1390–1371[a]		
AF 1013F	CTCGGAAACCGCAAGGACTG	*A. felis*	Ferredoxin	41–60[b]	632	7
AF 1013R	GATGTTGGGCCAAGTCTTAG	*A. felis*	Ferredoxin	635–672[b]		
Bhens probe	TGCGAGCATTTGGTTGGGCA	*B. henselae*	16S rRNA	1050–1069[c]		1
Afelis probe	TGCTACCATTTAGTTGAGCA	*A. felis*	16S rRNA	1073–1092[d]		1

[a] Positions within *Escherichia coli* 16S rRNA gene sequence (GenBank accession no. Eco 16s).
[b] Positions within *A. felis* ferredoxin gene sequence (GenBank accession no. X81826).
[c] Positions within *B. henselae* 16S rRNA gene sequence (GenBank accession no. M73229).
[d] Positions within *A. felis* 16S rRNA gene sequence (GenBank accession no. M65248).

upper-extremity scratches and a right supraclavicular enlarged lymph node. The node was minimally tender without erythema or local warmth and measured 2.0 by 1.5 cm. No inoculation papule was identified. Complete blood count, chemistry panel, sedimentation rate, and chest radiogram were within normal limits. No blood was left for serological testing, and the patient refused additional venipuncture. A skin test for CSD resulted in 2 mm of a palpable induration and erythema and was interpreted as negative. Fine-needle aspiration of the node was nondiagnostic. Four days later the puncture site began to drain purulent material. An excisional biopsy was performed. Histopathological examination showed reactive lymphadenitis with focal necrosis but without granulomata or microabscesses. Staining with Warthin-Starry silver stain was negative. Standard aerobic, anaerobic, mycobacterial, and fungal cultures were negative. The patient had an uneventful postoperative course and remained asymptomatic thereafter.

Isolation and characterization of *A. felis*. A portion of the excised lymph node was ground, cultured with brain heart infusion broth and agar, and incubated at 32°C without CO_2 and at 35°C with 5% CO_2. Two weeks later, fine growth was demonstrated in the broth incubated at 32°C, and grey colonies were noted on the agar plate. Subculture was performed on brain heart infusion and charcoal yeast extract agar plates. After several passages, the time to detection of growth was reduced to 5 days. Gram staining revealed small, thin, curved, gram-negative bacilli which were motile on wet mount. The biochemical profile of this microorganism included positive oxidase, urease, and nitrate reactions and negative catalase reactions. This biochemical profile was identical to that of the type strain of *A. felis*. The catalase and nitrate reactions distinguished this isolate from other *Afipia* species (Table 2).

PCR of the ferredoxin and the 16S rRNA genes. PCR amplification of the isolate's DNA with primers corresponding to the *A. felis* ferredoxin gene yielded, as expected, a single 632-bp band (Fig. 1). The identity of the isolate was also confirmed by PCR amplification of the 16S rRNA gene and hybridization with an *A. felis*-specific probe (Fig. 2, sample 6).

Cellular fatty acid analysis. Cellular fatty acid analysis showed the following composition: $C_{18:1\omega7C}$ (50%), $C_{16:1\omega7C}$ (6%), $C_{16:0}$ (4%), $C_{17:0cyc}$ (6%), $C_{16:0}2OH$ (4%), $C_{18:1\omega9C}$ (2%), $C_{18:0}$ (11%), $C_{19:0cyc\ \omega8C}$ (14%), and $C_{12:0}3OH$ (1%) and also a component with an equivalent chain length of 18.08, an unsaturated branched-chain fatty acid methyl ester (11-methyloctadec-12-enoic acid). The latter is a unique fatty acid found in the type strain of *A. felis* and characterized by gas-liquid chromatography and chromatography-mass spectrometry (21).

Sequencing of the 16S rRNA gene. In a pairwise comparison of the 16S rRNA gene sequence from the *A. felis* type strain (GenBank accession no. M65248) and AfTA-1, the two sequences were 99.86% similar (2 nucleotide differences of 1,391 nucleotides). When the type strain and AfTA-1 sequences were included in a multiple sequence alignment, edited to remove the variable regions at the 5′ and 3′ ends, and used as input for Phylip, *A. felis* ATCC 53690^T and AfTA-1 were found to be identical.

DNA relatedness studies. Labelled DNA from AfTA-1 was reacted with unlabelled DNAs from the type strains of *A. felis*, *A. clevelandensis*, and *A. broomeae* and reference strains for *Afipia* genomospecies 1, 2 and 3, as shown in Table 3. The

TABLE 2. Phenotypic characterization of the patient's isolate (isolate AfTA-1) and other *Afipia* strains

Strain	Test result				
	Oxidase	Catalase	Urease	Nitrate	Motility
AfTA-1	+	−	+	+	+
A. felis ATCC 53690^T	+	−	+	+	+
A. clevelandensis ATCC 49720Ta	+	−	+	−	+
A. broomeae ATCC 49717^T, ATCC 49718, and ATCC 49719[a]	+	w[b]	+	−	+
Afipia genomospecies 1 to 3[c]	+	w	+	−	+

[a] Results from Brenner et al. (9).
[b] w, weakly positive.
[c] Strains ATCC 49721, ATCC 49722, and ATCC 49723, respectively.

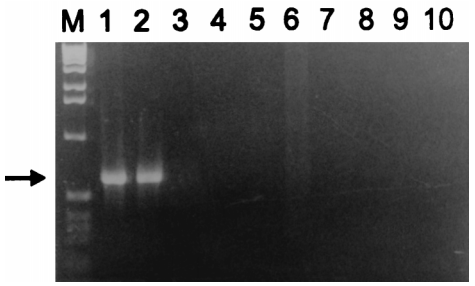

FIG. 1. Agarose gel electrophoresis of the ferredoxin gene PCR products. Lane M, 1-kb ladder size marker; lane 1, *A. felis* AfTA-1; lane 2, *A. felis* ATCC 53690^T; lane 3, *B. henselae* BhTA-2; lane 4, *B. henselae* BhTA-3; lane 5, *B. henselae* ATCC 49793^T; lane 6, sterile pus; lane 7, *S. aureus*; lane 8, *B. burgdorferi*; lane 9, *P. aeruginosa*; lane 10, water. The arrow indicates a 632-bp band of the PCR product.

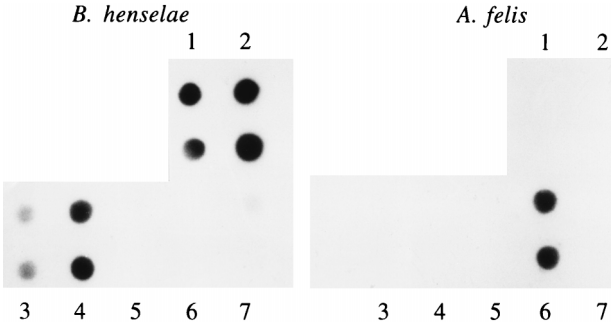

FIG. 2. The 16S rRNA PCR products were spotted, in duplicate, onto two identical membranes which were hybridized with either *A. felis*- or *B. henselae*-specific probes. 1, *B. henselae* DNA; 2 to 4, representative samples from CSD patients; 5, pus from a non-CSD patient; 6, *A. felis* AfTA-1 DNA; 7, water.

relatedness of AfTA-1 to the *A. felis* type strain was 99%, with 0.5% divergence within the related sequences.

Detection of *A. felis* and *B. henselae* DNAs in clinical specimens. In a recent study, we described 32 specimens (28 samples of pus and 4 samples of lymph node tissue) from 29 patients with typical clinical cases of CSD. These specimens were subjected to PCR for amplification of the 16S rRNA gene. All patients had regional lymphadenopathy and a history of contact with a cat(s). Hybridization identified *B. henselae* in all 32 specimens (6). To rule out dual infection with *A. felis* in the current study, aliquots of the PCR products were spotted in duplicate onto two identical membranes, and each membrane was hybridized with either a *B. henselae*- or an *A. felis*-specific probe. All specimens were positive for *B. henselae* DNA but negative for *A. felis* DNA. Representative results are presented in Fig. 2.

DISCUSSION

The phenotypic and genotypic characterizations as well as the DNA relatedness determination reported here leave no doubt that AfTA-1, isolated from a lymph node of a patient with clinical CSD, is a *A. felis*.

The isolation and characterization of *A. felis* from CSD patients have been extremely rare. English et al. (14) isolated the first *A. felis* strain (ATCC 53690^T) by direct culture on bacteriological media. Three other strains were isolated at the Centers for Disease Control and Prevention by a tissue culture method (9). To the best of our knowledge, AfTA-1 is the first *A. felis* strain cultured on standard bacteriological media since the first report of English et al. (14) in 1988 and the third report of the isolation of *A. felis* from the lymph nodes of CSD patients.

TABLE 3. DNA relatedness of strain AfTA-1 to type and reference strains of *Afipia* species

Source of unlabelled DNA	% Relatedness to labelled AfTA-1 DNA	% Divergence[a]
AfTA-1	100	0.0
A. felis ATCC 53690^T	99	0.5
A. clevelandensis ATCC 49720^T	26	
A. broomeae ATCC 49717^T	21	
Afipia genomospecies 1 (ATCC 49721)	25	
Afipia genomospecies 2 (ATCC 49722)	22	
Afipia genomospecies 3 (ATCC 49723)	20	

[a] A blank space indicates that the reaction was not performed.

Despite its rare isolation, indirect evidence suggests that *A. felis* may be more commonly linked to CSD than is currently appreciated. Drancourt et al. (13) reported on a patient with acute meningoencephalitis and a history of close cat contact who seroconverted to positivity for *A. felis* antibodies. In a serological study conducted in Italy among 80 patients with suspected classical or atypical CSD, 24 (30%) patients had antibodies against *B. henselae*, 10 (12%) had antibodies against *A. felis*, and 4 (5%) had antibodies against both organisms, as determined by an immunofluorescence assay (IFA) (15). In another study which examined the binding capacity of *B. henselae* and *A. felis* to peripheral blood lymphocytes of patients with clinical CSD, one of the five patients studied had elevated anti-*A. felis* antibody titers without anti-*B. henselae* antibodies, as determined by IFA (24). By a microagglutination test with heat-killed whole-cell antigen, 5 to 7% of 430 serum samples were reported to be positive for *A. felis* in one survey which studied the seroprevalence of antibodies against various *Afipia* strains (22). Amerein et al. (2) used an IFA to study serum samples from 35 CSD patients, 123 control patients without lymphadenopathy, 57 control patients with lymphadenopathy, and 102 nonpatient controls. Although no difference in *A. felis* antibody titers was found among these groups (contrary to the findings for anti-*B. henselae* antibody titers, which were significantly higher in the CSD group), 3 patients demonstrated high *A. felis* antibody titers. Two of these three patients belonged to the CSD group (representing 6% of the 35 CSD patients) and, in addition to anti-*A. felis* antibodies, also had high antibody titers to *B. henselae*.

Since both *A. felis* and *B. henselae* are members of the alpha-2 group of proteobacteria it would be reasonable to assume that common epitopes among these bacteria are responsible for cross-antigenicity. However, previous studies failed to demonstrate cross-reactivity between these two microorganisms, which differ both phenotypically and genotypically. Regnery et al. (25) have shown that high-titer human anti-*B. henselae* sera did not cross-react with *A. felis* antigen in the IFA and that hyperimmune rabbit antisera against *A. felis* did not react with *B. henselae* whole-cell antigen (25). Amerein et al. (2) could not demonstrate cross-reactivity between each of two hyperimmune rabbit antiserum samples against *A. felis* and *B. henselae* and the heterologous antigen. Due to the apparent lack of cross-reactivity between *A. felis* and *B. henselae*, the demonstration of simultaneous seropositivity to both organisms suggests that coinfection with both *A. felis* and *B. henselae* may occur in at least a small minority of patients with CSD. Although *A. felis* and *B. henselae* have never been cocultivated from the same clinical specimen, they were coidentified by PCR in 2 of 12 lymph node specimens from CSD patients (1). We did not identify *A. felis* DNA in any of the 32 specimens which were PCR positive for *B. henselae* DNA. This observation is in accordance with that of other studies (4, 7, 16), suggesting that if coinfection does occur, it is rare.

Also, when the diagnosis of CSD is based solely on histopathology, some cases could be wrongly interpreted as being caused by *B. henselae* instead of *A. felis* since both microorganisms are morphologically indistinguishable and both stain well with the Warthin-Starry silver stain (14, 20).

In conclusion, evidence based on serological studies or DNA amplification suggest that *A. felis* may play a role in the pathogenesis of CSD, either as a single pathogen or in conjunction with *B. henselae*. These data are difficult to interpret and are inconclusive. However, the culture-confirmed cases of CSD caused by *A. felis* described by English et al. (14) and Brenner

et al. (9), together with the case of CSD caused by *A. felis* in the patient described in this report, implicate *A. felis* as a rare cause of CSD. If attempts at the diagnosis of CSD continue to be directed only at the identification of *B. henselae*, as was the case in several recent studies (4, 20, 23, 28), and not at *A. felis* as well, the diagnosis of CSD might be missed for those patients with CSD caused by *A. felis*.

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