**Bartonella henselae** Associated with Parinaud’s Oculoglandular Syndrome

Danilla Grando, Laurence J. Sullivan, James P. Flexman, Mark W. Watson, and John H. Andrew

_Bartonella henselae_ was recovered from the conjunctival scraping of a 38-year-old woman who presented with a 2-week history of tender preauricular lymphadenopathy and a 1-day history of a red left eye. Dry adherent colonies were observed on agar plates at 21 days of incubation, and the isolate was identified through conventional and molecular tests. Polymerase chain reaction (PCR) amplification of a specific region of the 16S rRNA gene and confirmation by a separate PCR reaction with hybridization of the product with a _B. henselae_-specific probe confirmed the isolate as _B. henselae_. This is the first reported isolation of the causative agent of cat scratch disease from ocular tissue in a patient with Parinaud’s oculoglandular syndrome.

Parinaud’s oculoglandular syndrome (POGS) is a form of conjunctivitis that can accompany other overt symptoms of cat scratch disease (CSD). It has been documented to occur in up to 6% of patients with this disease [1, 2]. The conjunctivitis is unilateral and nonsuppurative, with an area of focal granulomatous reaction. It is associated with regional lymphadenopathy that predominantly affects either the preauricular or submandibular lymph nodes.

There is mounting evidence that the principal cause of CSD is infection with _Bartonella henselae_ [3–5] following contact with cats [6]. Prolonged bacteremia with _B. henselae_ has been demonstrated in domestic cats [7–9], and this is thought to act as a reservoir of infection. It is probable that cat fleas play a role in the transmission of disease [8, 10, 11]. CSD is a subacute illness, usually characterized by regional granulomatous lymphadenitis and variable systemic symptoms that may include fever [1, 2, 12]. A papule may occur at the site of inoculation and can be located in 50%–90% of patients. POGS is thought to occur after inoculation of the eye, and there have been reports of complications such as optic neuritis and encephalopathy following the lymphadenopathy. This case report describes the first published isolation of _Bartonella_ species from a patient with POGS and supports evidence that _B. henselae_ is the principal causative agent of CSD.

**Case Report**

A 38-year-old woman from rural New South Wales, Australia, presented to the Royal Victorian Eye and Ear Hospital (Melbourne) after a red eye had developed the previous day, after a 2-week history of preauricular lymphadenopathy. She was unable to recall being scratched by her cat. Chloramphenicol eye drops had been prescribed on the previous day by her family doctor. On examination, her left eye was mildly injected with moderate subtarsal papillae and a few large follicles. The left superior bulbar conjunctiva was notable for a large area of granulomatous tissue with ulceration and underlying episcleritis. The eye was not tender, and she had severe left preauricular lymphadenopathy. The patient was afebrile.

A scraping from the ulcerated conjunctiva was inoculated directly onto horse blood and chocolate agar plates, and smears were prepared for microbiological investigation. Then the patient began receiving oral amoxicillin/clavulanate (1 g q.i.d.) and doxycycline (100 mg q.d.). Conjunctival drops (tobramycin and chloramphenicol) were administered every 2 hours. A conjunctival biopsy was done the following day. On histological examination, marked follicular conjunctivitis with epithelial infiltration by neutrophils and microcystic edema were noted. No granulomatous inflammation was seen, although a number of plasma cells were present. Gram, Ziehl-Neelsen, periodic acid–Schiff, and Warthin-Starry silver staining for organisms in tissue sections all yielded negative results. Results of a full blood examination and the patient’s erythrocyte sedimentation rate were normal. Rheumatoid factor, anti-nuclear antibody, anti-cardiolipin antibody, and anti-neutrophil cytoplasmic antibody were not detected. Acute-phase serological study by use of an indirect immunofluorescence antibody test [13] demonstrated IgG antibodies to _B. henselae_ at a titer of 512. A titer $\geq 64$ has been suggested as highly predictive of CSD [13].

The lymphadenopathy had diminished at 3 days, but retinal changes (cotton wool spots and a small white intraretinal lesion) were observed. Such retinal lesions have been reported in association with CSD [14]. These changes resolved over the following 3 weeks, as did other external signs.

**Methods**

After receipt in the laboratory, the horse blood agar and chocolate blood agar plates were incubated at 37°C in an atmo-
sphere containing 5% CO₂. The prepared smear was gram-stained; 0.25% safranin O was used as counterstain. Numerous polymorphs and pale-staining, slightly curved, gram-negative pleomorphic bacteria were present. Francisella tularensis has been implicated as a cause of POGS [15], although it has never been isolated in Australia. Hence, it was decided to prepare an additional chocolate blood agar plate supplemented with 1% IsoVitaleX (BBL Becton Dickinson Microbiology Systems, Cockeysville, MD) [16]. Some of the excess material inoculated onto the horse blood agar was then transferred to the supplemented chocolate agar inside a class II biohazard cabinet for protection. The plates were then sealed with plastic film, and small holes were made in the film to allow perfusion of CO₂. The plates were returned to the CO₂ incubator and examined daily for 1 week and weekly thereafter.

At 3 weeks, a total of ~12 small (1 mm), translucent, dry, adherent rough colonies were present in the primary inoculum of the two chocolate blood agar plates. Gram-staining of the colonies again demonstrated pale-staining pleomorphic bacilli. Initial tests showed the organism to be oxidase- and catalase-negative, and motility could not be demonstrated in either 4-hour or overnight broth cultures. Colonies were visible at 72 hours on subculture. We were unable to demonstrate X and V factor dependence, but there was a slight suggestion of satellitism around a Staphylococcus aureus streak on horse blood agar. Colonies on subculture showed variation in colonial morphology between smooth and rough. By use of heavy suspensions of these subcultures, various biochemical systems were inoculated. Use of MicroScan Rapid Anaerobe Identification panels (Baxter Diagnostics, Deerfield, IL) gave a profile number 10077640, which was indistinguishable from the profile obtained with a Bartonella quintana type strain (American Type Culture Collection VR-358). Use of RapID ANA II System panels (Innovative Diagnostic Systems, Norcross, GA) gave a profile number 000671, which is consistent with that reported by others [17] and was identical to that obtained for the B. quintana type strain.

A specific region of the 16S rRNA gene was amplified by use of a PCR based on the p12B and p24E primers designed by Relman et al. [18]. The 296-bp product contains seven base differences between B. henselae and B. quintana. The product was sequenced in both directions on an Applied Biosystems 373A automated DNA sequencer that used dye-terminator chemistry. The isolate was found to be identical to the reference strain of B. henselae (GenBank accession number M73229) in the region examined. The isolate was subsequently confirmed as B. henselae by use of a separate set of primers that amplify a 414-bp product and hybridization with an oligonucleotide probe specific for B. henselae [3].

Discussion

The isolation of B. henselae from clinical material has been difficult to achieve. In this case, a large number of organisms were seen in the gram-stained sample, yet only 12 colonies were isolated from macroscopically evident material on agar plates. Although this is the first published report of isolation of B. henselae from POGS, it is possible that the same organism was isolated from ocular material as far back as 1933 [19]. Earlier, Verhoff [20] had demonstrated histologically the presence of organisms in ocular tissue from patients with POGS. The modified ‘gram’ stain used by Verhoff [21] rendered the organism gram-positive; however, his description of the cultural characteristics of the organism are strikingly similar to those described above. Between 1913 and 1933, Verhoff was able to demonstrate histologically the presence of the organism he termed Leptothrix in the ocular tissue of 45 of 46 patients with POGS [19]. In 1933, Verhoff and King [19] described being able to grow Leptothrix in pure culture from 3 of 4 patients with POGS. Although sporadic reports of isolation of organisms continued to occur until the 1940s, the term Leptothrix, along with reports of its isolation, disappeared from the literature. From the 1950s, reviewers of the literature on CSD considered these early reports of Leptothrix isolation to be due to contamination of cultures, rather than evidence of an etiologic agent.

Parinaud’s ocular glandular syndrome was first described by Henri Parinaud in 1889 [22]. An association between POGS and CSD was not made until 1953 [23]. B. henselae has since been isolated from the lymph nodes of patients with CSD [24]. This isolation of Bartonella species confirms that the ocular manifestations of POGS are indeed due to the same agent responsible for CSD. Relman [25] has stated that 16S rRNA sequence identity between two organisms may be insufficient to confirm that the organisms belong to the same species. However, a PCR assay has been described in which the product can be used as a target for species-specific oligonucleotide hybridization probes that differentiate B. henselae from B. quintana [3]. This method was used in this case to confirm the isolate as B. henselae.

Cultivation of B. henselae has proven to be difficult and cannot be used reliably to diagnose CSD. We were unable to demonstrate the presence of organisms in histological sections. Detection of organisms in stained sections of conjunctival tissue from POGS may be unreliable [19, 26]. Recently, PCR has been used to detect Bartonella DNA in tissue [3, 4, 27], but this may require an invasive procedure to obtain biopsy material for testing. Serological studies may be too insensitive in some patients to lead to a reliable diagnosis [28]. In addition, serological results must be interpreted with care, as asymptomatic patients may be positive for IgG antibody to Bartonella species [6, 28, 29]. Unfortunately, it was not possible to obtain a convalescent specimen of serum from our patient. Currently, the diagnosis of CSD is based on a typical clinical presentation, with a titer of IgG antibody to Bartonella species of ≥64 as measured by immunofluorescence [5, 6].

If indeed Verhoff had previously isolated Bartonella species from conjunctival tissue, then it is remarkable that further isola-
tions have not been made in the last 50 years. This paper serves to illustrate that with adequate collection techniques and the provision to extend culture times beyond those routinely used in laboratories, further isolations of *Bartonella* species should be possible.

### References