CASE REPORT

Life-threatening angioedema of the tongue: the detection of the RNA of *B henselae* in the saliva of a male patient and his dog as well as of the DNA of three *Bartonella* species in the blood of the patient

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SUMMARY

Non-hereditary angioedema is a common disease with a prevalence between 5% and 19% and approximately half of the patients experience a swelling of the tongue. We report a case of a 49-year-old Caucasian man with a gross life-threatening angioedema of the tongue, whose attacks occurred every 4 weeks. The most frequent causes of angioedema were excluded. We detected DNA and RNA from *Bartonella henselae* in the blood and saliva of the patient and in the saliva of the patient's hunting dog. Treatment with azithromycin plus minocycline cleared the blood and saliva of RNA and DNA of *Bartonella* species, and the patient has been free from angioedema for 1 year. None of the therapy modalities used to treat the hereditary form or ACE or allergy-induced angioedema affect the detrimental course caused by *Bartonella* species. We therefore suggest that a molecular *Bartonella* test be included in the analysis of angioedema.

BACKGROUND

Non-hereditary angioedema is a common disease with a prevalence between 5% and 19%, according to epidemiological studies, whereas hereditary angioedema has a prevalence of 0.0014%.¹ Angioedema presents with swelling of the cutaneous or mucous membranes and frequently involves the lips, eyes, neck, arms, legs and throat. Approximately 50% of patients with angioedema experience a swelling of the tongue. A frequent cause of the angioedema of the tongue is a quantitative or functional defect in the complement system of the C1 esterase inhibitor. To the best of our knowledge, no association between angioedema and *Bartonella* infection has been reported so far.

Bartonella species and *Bartonella henselae* have emerged as important infectious pathogens in many diseases. Classically, patients infected with *B henselae* present with cat scratch disease, typically accompanied by regionally swollen lymph nodes which is called lymphadenopathy. Atypical symptoms are granulomatous conjunctivitis, optic neuritis, bacillary angiomatosis (in immune-compromised patients) and acute encephalopathy. The life cycle, as well as the cellular targets of *Bartonella* species, indicates that *Bartonella* species can cause a variety of symptoms and diseases. *Bartonella* species are facultative intracellular bacteria that mainly infect erythrocytes and endothelial cells. In humans,

transmission generally occurs through arthropod or animal bites and scratches. More than 30 different *Bartonella* species have been identified in small mammals.² At present, 12 *Bartonella* species are recognised or suspected to be human pathogens.³ *B henselae* is the most frequent pathogen in humans, and cats are the main zoonotic reservoir of *B henselae*, as shown in serological and PCR-based studies.^{4–6} Vectors such as fleas and ticks commonly transmit the infection between cats, and it is less often transmitted to humans. We present an unusual case of gross angioedema of the tongue in a patient infected with *B henselae* and *Bartonella elizabethae*.

CASE PRESENTATION

A healthy-looking 49-year-old man suffered from recurrent gross swelling of the tongue. During the angioedema attacks, he also noticed a swelling of his hands and arms. Two years prior to presentation, the attacks occurred every 4 weeks with decreasing intervals. At the time of presentation, the patient suffered from these attacks every second week and felt as if he was being suffocated. The efficiency of various medications (etoricoxib, gabapentin) has decreased over time.

The family history did not indicate a familial burden for angioedema. Both parents are alive and in reasonable condition. The patient's brother, his 7-year-old child and his wife are all healthy. He complained about frequent respiratory infections between January and July 2010.

The patient's only pet is a hunting dog. The patient noticed that he did not experience an attack during a 4-week vacation without this dog. In 2010 and 2011, hospital doctors carried out careful physical examinations as well as thorough biochemical, physiological and radiological screening tests. Only minimal deviations from the normal values were observed. All of the blood tests were normal, including tests for complement factors C1q and C4, and the quantitative and functional measurements of the C1q inhibitor because deficiencies in the complement system can be the cause of angioedema. ELISA IgG and ELISA IgM against *Borrelia burgdorferi* were negative.

Our screening aimed to identify an infectious agent, to determine the proper drug treatment and to explore the conditions for an adoptive cell therapy, which requires the absence of certain viral infections. Serological ELISA tests for hepatitis A, B

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and C, HIV, cytomegalovirus, and human herpesvirus-6 were negative, analyses of the herpes simplex virus (HSV1), HSV2 and Epstein-Barr virus antibodies showed no signs of an acute or active recurring infection. A low-grade IgG antibody titre against *Toxoplasma gondii* indicated a previous infection. No antibodies were detected against *Chlamydia* spp. The patient showed no antibody responses against *B henselae*.

Molecular analyses were performed on samples of blood, saliva and stool collected from the patient and his wife, blood and stool collected from the son, and stool and saliva collected from the dog. The molecular results for *Bartonella* spp. and *Helicobacter* spp. are shown in table 1.

Genomic DNA from 1×10^6 peripheral blood mononucleated cells and saliva samples from the patient and his relatives was obtained using the Quick Gene DNA Whole Blood Kit (Kurabo, Japan). Using the PSP Spin Stool DNA Plus Kit of Stratec Molecular (Germany), DNA was extracted from the stool samples according to manufacturer's instructions. Complete RNA was isolated using the Qiagen RNeasy Mini Kit (Qiagen, Germany), cDNA was generated using random hexamer primers and the RevertAid First Strand Synthesis Kit (Thermo Scientific, Germany). The DNA and cDNA were analysed using the non-quantitative real-time PCR-based detection methods published by Pantchev *et al*,⁷ Miyashita *et al*,⁸ and Chen *et al*.⁹ The results were verified using the published nested PCR methods to identify current Chlamydiaceae infection.¹⁰ In parallel, we used DNA from *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia psittaci* as control samples, and we also included a non-template control. We investigated the samples for the presence of *Bartonella* spp. infection using the non-quantitative real-time PCR-based method of Maggi *et al*¹¹ and Breitschwerdt *et al*.¹² Positive results are verified using the method published by Jensen *et al*,¹³ which allows for discrimination, but not quantification, of *Bartonella clarridgeiae*, *Bartonella quintana*, *B henselae*, *Bartonella bacilliformis*, *Bartonella elizabethae*, *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella grahamii*. In parallel, we used the DNA of *B henselae* as a control sample, and we included a non-template control. Our efforts to minimise cross-contamination included the physical separation of all PCR steps and the use of interspersed negative controls. For the investigation of *Helicobacter hepaticus* we used the method of Ge *et al*¹⁴ and Ito *et al*¹⁵ that was established to detect *Helicobacter pylori*. For the detection of *Helicobacter felis*, we followed the method

of Begue *et al*.¹⁶ To verify the positive results, we performed a second round of investigation using the method published by Chisholm *et al*,^{17 18} which allows for the discrimination, but not quantification, of *H pylori*-related and *Helicobacter heilmannii*-related organisms (such as *H heilmannii* and *H felis*). In parallel, we used DNA from *H pylori*, *H hepaticus* and *H felis* as control samples, and we included a non-template control. To confirm the results after the first PCR analysis, the PCR was repeated at least once. All samples were verified as positive by the second PCR. To obtain PCR amplification for sequencing, individual rather than multiplex PCRs were used. The Sequencing Service (SeqService) of the Department of Biology, University of Munich, Germany, sequenced all of the amplification products for the positive samples (defined as the presence of detectable amounts of DNA) to verify the specificity of the PCR products.

TREATMENT

Treatment with azithromycin (500 mg daily for 3 days, repeated after 7 days) plus minocycline (50 mg daily for 2 weeks) cleared the blood and saliva of the RNA and DNA of all three *Bartonella* species.

OUTCOME AND FOLLOW-UP

Until publication of this article, the patient stayed in good condition and showed no sign of recurrence.

DISCUSSION

To the best of our knowledge, this is the first report associating *Bartonella* species with a gross angioedema of the tongue. *Bartonella* species have been described as cause of a variety of diseases but so far not of angioedema of the tongue, neither in animals nor in men. Dogs are less frequently infected with *B henselae* than cats. A PCR-based study in California of 103 shelter cats and 120 dogs detected *Bartonella* in 21 cats but only in 2 dogs.¹⁹ A study in North Carolina detected *Bartonella* DNA in 61 of 663 dogs, and *B henselae* DNA was identified in 30 of the 61 dogs.²⁰ There is considerable regional variability in the prevalence of fleas infected with *Bartonella* spp.²¹ In 952 fleas collected from 148 cats and 133 dogs, 9.5% of the French dog fleas, but none of the German dog fleas, were positive for *Bartonella* DNA.²¹

A frequent cause of the angioedema of the tongue is a quantitative or functional defect in the complement system of the C1 esterase inhibitor. Eradication of *Helicobacter* can essentially improve the symptoms of the hereditary angioedema.^{22 23} In our patient, no defect could be detected in the complement system of the C1 esterase inhibitor. Furthermore, the patient did not receive drugs that may have caused angioedema of the tongue, such as the antihypertensive modality ACE inhibitor, or antiepileptic drugs, such as phenytoin.²⁴ *Helicobacter*, which can induce angioedema, was detected in the blood and the stool of the patient's wife and his son, but it was not detected in the patient's blood or stool (data not shown).

The blood sample from the patient's son was free of *B henselae* RNA and DNA, and his saliva was not tested. *B henselae* RNA was detected in the blood of the patient's wife, but not in her saliva (table 1). The patient's wife and son had no symptoms of angioedema. Taken together, the *Helicobacter* tests of the patient were negative, but the RNA of *B henselae* was detected in the patient's blood and saliva (table 1). This finding strongly suggests that *B henselae* is responsible for the angioedema of the tongue. It is unclear whether the DNA of *B elizabethae* and *B bacilliformis* interfered with the immune response, either by suppressing the antibody response or enhancing the inflammatory angioedema

Table 1 Real-time PCR amplification of *Bartonella* species in a patient with angioedema and his family

	Patient		Wife		Son		Dog	
	Blood	Saliva	Blood	Saliva	Blood	Saliva	Blood	Saliva
<i>Bartonella henselae</i>								
DNA	+	+	+	–	–	n.t.	n.t.	+
RNA	+	+	+	n.t.	n.t.	n.t.	n.t.	+
<i>Bartonella elizabethae</i>								
DNA	+	–	–	–	–	n.t.	n.t.	–
RNA	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Bartonella bacilliformis</i>								
DNA	+	–	–	–	–	n.t.	n.t.	–
RNA	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

+, DNA or RNA of the respective *Bartonella* species was detected. Positive results of DNA samples were confirmed by sequence analysis.

–, no DNA or RNA was detected.

n.t., Not tested.

reaction by *B henselae*. Only one report exists of *B elizabethae* as a human pathogen causing bacteraemia and endocarditis.²⁵ One can speculate whether *B elizabethae* caused or contributed as a pathogenic factor to the development of the patient's hypermobile interatrial septum.

Learning points

- ▶ *Bartonella* species can be relevant pathogens in angioedema of the tongue.
- ▶ Therefore, and since serological analyses can be misleading, a molecular *Bartonella* test should be included in the analysis of angioedema (we recommend real-time PCR and reverse transcription real-time PCR to detect bacterial DNA and RNA).
- ▶ Treatment with azithromycin (500 mg daily for 3 days, repeated after 7 days) plus minocycline (50 mg daily for 2 weeks) should be sufficient to clear the *Bartonella* species infection.

Contributors The patient was presented to RW who is responsible for treatment and overall guidance. BL is responsible for laboratory diagnosis.

Competing interests None.

Patient consent Obtained.

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REFERENCES

- Madsen F, Attermann J, Linneberg A. Epidemiology of non-hereditary angioedema. *Acta Derm Venereol* 2012;92:475–9.
- Gil H, Garcia-Esteban C, Barandika JF, et al. Variability of *Bartonella* genotypes among small mammals in Spain. *Appl Environ Microbiol* 2010;76:8062–70.
- Slater NL, Welch DF. *Bartonella*, including cat-scratch disease. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and practice of infectious diseases*. Churchill Livingstone: Elsevier, 2010.
- Anderson B, Sims K, Regnery R, et al. Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J Clin Microbiol* 1994;32:942–8.
- Avidor B, Kletter Y, Abulafia S, et al. Molecular diagnosis of cat scratch disease: a two-step approach. *J Clin Microbiol* 1997;35:1924–30.
- Scott MA, McCurley TL, Vnencak-Jones CL, et al. Cat scratch disease: detection of *Bartonella henselae* DNA in archival biopsies from patients with clinically, serologically, and histologically defined disease. *Am J Pathol* 1996;149:2161–7.
- Pantchev A, Sting R, Bauerfeind R, et al. New real-time PCR tests for species-specific detection of *Chlamydomydia psittaci* and *Chlamydomydia abortus* from tissue samples. *Vet J* 2009;181:145–50.
- Miyashita N, Obase Y, Fukuda M, et al. Evaluation of the diagnostic usefulness of real-time PCR for detection of *Chlamydomydia pneumoniae* in acute respiratory infections. *J Infect Chemother* 2007;13:183–7.
- Chen CY, Chi KH, Alexander S, et al. A real-time quadruplex PCR assay for the diagnosis of rectal lymphogranuloma venereum and non-lymphogranuloma venereum *Chlamydia trachomatis* infections. *Sex Transm Infect* 2008;84:273–6.
- Fellerhoff B, Laumbacher B, Mueller N, et al. Associations between *Chlamydomydia* infections, schizophrenia and risk of HLA-A10. *Mol Psychiatry* 2007;12:264–72.
- Maggi RG, Harms CA, Hohn AA, et al. *Bartonella henselae* in porpoise blood. *Emerg Infect Dis* 2005;11:1894–8.
- Breitschwerdt EB, Hegarty BC, Maggi R, et al. *Bartonella* species as a potential cause of epistaxis in dogs. *J Clin Microbiol* 2005;43:2529–33.
- Jensen WA, Fall MZ, Rooney J, et al. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. *J Clin Microbiol* 2000;38:1717–22.
- Ge Z, White DA, Whary MT, et al. Fluorogenic PCR-based quantitative detection of a murine pathogen, *Helicobacter hepaticus*. *J Clin Microbiol* 2001;39:2598–602.
- Ito T, Kobayashi D, Uchida K, et al. *Helicobacter pylori* invades the gastric mucosa and translocates to the gastric lymph nodes. *Lab Invest* 2008;88:664–81.
- Begue RE, Manning J, Correa H. Real-time PCR for quantification of *Helicobacter felis* in mouse stomach. *South Med J* 2006;99:1306–7.
- Chisholm SA, Owen RJ. Development and application of a novel screening PCR assay for direct detection of 'Helicobacter heilmannii'-like organisms in human gastric biopsies in Southeast England. *Diagn Microbiol Infect Dis* 2003;46:1–7.
- Chisholm SA, Owen RJ, Teare EL, et al. PCR-based diagnosis of *Helicobacter pylori* infection and real-time determination of clarithromycin resistance directly from human gastric biopsy samples. *J Clin Microbiol* 2001;39:1217–20.
- Tsai YL, Lin CC, Chomel BB, et al. *Bartonella* infection in shelter cats and dogs and their ectoparasites. *Vector Borne Zoonotic Dis* 2011;11:1023–30.
- Perez C, Maggi RG, Diniz PP, et al. Molecular and serological diagnosis of *Bartonella* infection in 61 dogs from the United States. *J Vet Intern Med* 2011;25:805–10.
- Just FT, Gilles J, Pradel I, et al. Molecular evidence for *Bartonella* spp. in cat and dog fleas from Germany and France. *Zoonoses Public Health* 2008;55:514–20.
- Farkas H, Fust G, Fekete B, et al. Eradication of *Helicobacter pylori* and improvement of hereditary angioneurotic oedema. *Lancet* 2001;358:1695–6.
- Visy B, Fust G, Bygum A, et al. *Helicobacter pylori* infection as a triggering factor of attacks in patients with hereditary angioedema. *Helicobacter* 2007;12:251–7.
- Mondal R, Sarkar S, Sabui T, et al. Phenytoin induced life threatening macroglossia in a child. *J Neurosci Rural Pract* 2013;4:75–7.
- Daly JS, Worthington MG, Brenner DJ, et al. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. *J Clin Microbiol* 1993;31:872–81.

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