Cat-bite-induced *Francisella tularensis* infection with a false-positive serological reaction for *Bartonella quintana*

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**Abstract**

**Introduction.** Tularemia is caused by infection with *Francisella tularensis* transmitted via direct contact with an infected hare carcass or through the bites of vectors, such as mosquitoes or ticks [1]. Infected cats are known to transmit the disease to humans [2, 3], and bites by infected squirrels and hamsters have also been reported to cause tularemia [4]. The disease may also be contracted by inhalation or by ingestion of infected water or food. In Sweden, the majority of tularemia cases occur during late summer and early autumn in the ulceroglandular form [5, 6]. A typical case would be a patient who developed a primary lesion after a mosquito bite on the leg, with high fever and engagement of the proximally located inguinal lymph nodes [2].

Here, we present a case of ulceroglandular tularemia infection after a cat bite, with a false-positive reaction in the serological test for *Bartonella quintana* (the causative agent of trench fever). The case is enlightening about the importance of extending the medical history and re-sampling the patient for antibody detection when the clinical suspicion of cat-bite-associated tularemia is high. The false-positive result for anti-*B. quintana* antibodies may have been due to technical issues with the assay, cross-reactivity or both.

**INTRODUCTION**

Tularemia, a zoonosis caused by the small Gram-negative bacterium *Francisella tularensis*, is caused mainly via transmission from direct contact with an infected hare carcass or through the bites of vectors, such as mosquitoes or ticks [1]. Infected cats are known to transmit the disease to humans [2, 3], and bites by infected squirrels and hamsters have also been reported to cause tularemia [4]. The disease may also be contracted by inhalation or by ingestion of infected water or food. In Sweden, the majority of tularemia cases occur during late summer and early autumn in the ulceroglandular form [5, 6]. A typical case would be a patient who developed a primary lesion after a mosquito bite on the leg, with high fever and engagement of the proximally located inguinal lymph nodes [2].

On January 14th 2015, a previously healthy 56-year-old man was examined by a general practitioner in Örebro, Sweden, due to the patient having a fever after a cat bite on his right thumb 19 days earlier. A small pustule had appeared on the thumb and had developed into a painless ulcerated lesion. During the preceding 10 days, he had experienced pain in his right axilla and a fever of 39.5 °C. The general practitioner suspected cat-scratch fever, caused by *Bartonella henselae*, and referred the patient to the Department of Infectious Diseases, Örebro University Hospital, Örebro, Sweden, for further examination.

At the hospital, the initial investigation revealed a rectal temperature of 38.2 °C and a heart rate of 100 beats min⁻¹. The patient had an erythema of 12 × 15 mm with peeling skin on his right thumb, and an ulcer of 5 × 5 mm in the centre. He had a tender 20 mm lymph node in his right axilla. On his right upper arm, extending from the medial side of his elbow to his axilla, a mild lymphangitis was observed. No other abnormal findings were noted after examination of his lungs, skin and other regional lymph nodes. He had an elevated white blood cell count of 11.9×10⁹ cells l⁻¹ (reference value 3.5–8.8×10⁹ cells l⁻¹), a C-reactive protein level of 23 mg l⁻¹.

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**CASE REPORT**

**Introduction.** Tularemia is caused by infection with *Francisella tularensis* transmitted via direct contact with an infected hare carcass or indirectly through the bites of vectors, but may be cat-bite-associated as well. Medical history and reliable diagnostic analysis are important in order to differentiate it from other cat-associated infections, e.g. *Bartonella* spp.

**Case presentation.** A healthy 56-year-old man was examined because of a cat-bite-associated ulceroglandular wound on his right thumb. Nineteen days after the cat bite occurred, a serology test was positive for anti-*F. tularensis*, but negative for anti-*F. tularensis*. Since *Bartonella* infections are rare in Sweden, another serology test was analysed 2 weeks later with a positive result for anti-*F. tularensis*. The patient was treated with doxycycline for 14 days and recovered. The patient was re-sampled after 18 months to obtain a convalescent sample. The acute and the convalescent samples were both analysed at a reference centre, with negative results for anti-*Bartonella* spp. this time.

**Conclusion.** This case is enlightening about the importance of extending the medical history and re-sampling the patient for antibody detection when the clinical suspicion of cat-bite-associated tularemia is high. The false-positive result for anti-*B. quintana* antibodies may have been due to technical issues with the assay, cross-reactivity or both.
The laboratory bodies. Treatment was then initiated with doxycycline, 200 mg once a day for 2 weeks. At the follow-up contact, by telephone after 7 days, the patient was completely recovered. The laboratory’s request form for anti-Bartonella spp. immunoglobulins included testing for both anti-B. henselae antibodies and anti-B. quintana antibodies. The assays were performed by indirect immunofluorescence test (Euroimmun kit) for IgG and showed a negative result for anti-B. henselae (IgG titre <1:64), but a positive result for anti-B. quintana (IgG titre 1:128). Briefly, the assays were performed manually by incubation of human serum on a glass slide together with fluorescent secondary antibodies and the results were evaluated by fluorescence microscopy. The serological analysis for anti-F. tularensis antibodies, performed with a tube agglutination test (Widal reaction), was negative (IgM/IgG titre <1:64).

Since B. quintana infections are rare in Sweden, the diagnosis was considered unlikely and the patient was contacted again by telephone. He explained that the cat was brought to his recreational (holiday) cottage 2 weeks before the cat bite occurred, and a dead hare had been found in the cottage garden where the cat had its territory. Since the time of exposure, the cat had not shown any signs of disease; it was later examined by a veterinarian, but had no signs of tularaemia nor louse infection. On suspicion of ulceroglandular tularaemia indirectly transmitted by the cat’s saliva, a second serum sample for anti-F. tularensis antibodies was taken 32 days after the cat bite occurred. This time the assay was positive (IgG titre 1:320) and a final diagnosis of tularaemia was made. The positive serological test for anti-B. quintana antibodies was considered as a false-positive result.

Since B. quintana may be a chronic infection and antibodies may persist over many years [7], we resampled the patient 19 days after the cat bite occurred and analysed for antibodies and anti-B. quintana immunoglobulins included testing for both anti-B. henselae and anti-B. quintana antibodies. The samples were again negative for anti-B. henselae (IgG titres <1:100 and <1:100, respectively) and anti-B. quintana (IgG titres <1:100 and <1:100, respectively) antibodies by using a microimmunofluorescence assay, as described elsewhere [8, 9].

DISCUSSION

Tularaemia is associated with a variety of clinical manifestations similar to those seen in other vector-borne infections [10]. Cat-associated tularaemia is uncommon, but has been reported before in Sweden [6] and other countries [11]. To distinguish tularaemia from other infections may be difficult and requires a detailed medical history from the patient, and sensitive and specific laboratory methods, as well as a re-sampling of the the patient when the diagnosis is unclear. F. tularensis cultivation should be avoided due to the risk of laboratory transmission [12] and as the bacterium does not grow in routinely plated cultures [13]. PCR applied on samples from primary lesions is a fast and sensitive method [12]. The most common method used for diagnosing tularaemia is by detection of antibodies in serum by agglutination or enzyme-linked immunosorbent assays [13, 14].

Diagnosing tularaemia by antibody detection has several limitations. Firstly, IgM and IgG appear together, but are often not detectable until 2 to 3 weeks after infection [15, 16]. If the first serology is analysed too early, a negative result may be fallacious and the patient should be resampled after a few weeks. Secondly, cross-reactions have been described between F. tularensis and species of Brucella, Proteus and Yersinia [12, 16]. Moreover, false-positive and false-negative serological test results may occur due to technical problems with the assays or inadequate performance of an assay by the technician.

In this report, the positive titre for anti-B. quintana antibodies (1:128) was unexpected, since clinical infections with the bacteria are uncommon [17, 18] and it has not been associated with cat bites previously in Sweden [19, 20]. Infections caused by B. quintana are exclusively seen in patients with actual or recent contact with body lice [21]. In this case, neither the patient nor the cat had been exposed to lice. Also, in Sweden the seroprevalence of anti-B. quintana antibodies is very low among humans [22, 23] and cats [24].

B. quintana has been reported to cross-react with the genus Bartonella [25, 26], and with species of Chlamydophila [27, 28] and Coxiella [9]. In clinical samples, cross-reactivity between F. tularensis and B. quintana has not been described to our knowledge. However, it is known that F. tularensis and Bartonella spp. express common proteins that may elicit an antibody response in infected individuals [29]. In a previous study by Gilmore et al. [30], rabbit anti-F. tularensis antibodies reacted with a recombinant immunogenic protein (SucB) derived from B. quintana. In
another study, Litwin et al. [31] showed that a recombinant SucB protein from B. henselae cross-reacted with human serum containing anti-F. tularensis antibodies. The B. henselae sucB gene used in the study was determined to have 85.3% identity to the SucB protein of B. quintana. These findings indicate that cross-reactions between F. tularensis and B. quintana are possible in immunological assays on clinical samples.

In this report, a significant titre of anti-B. quintana antibodies was detected by the Euroimmun assay in the initial serum sample, but not when the sample was re-analysed, and it was negative by the microimmunofluorescence assay. This may be explained by technical challenges in the performance of the Euroimmun assay and/or by differences in the specificities of the two assays. A limitation of this study was that we did not evaluate the two assays with multiple samples from F. tularensis-positive patients and we did not re-evaluate the serum samples after neutralization of anti-F. tularensis antibodies to evaluate positive results for anti-B. quintana antibodies. Therefore, we suggest that future evaluations of immunoasays for anti-F. tularensis antibodies should include specificity analysis of cross-reactivity with anti-B. quintana antibodies and that clinicians should be aware of potentially false-positive results in tularemia patients.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This case report was written with permission from the patient, and in accordance with the Declaration of Helsinki and the ethical standards of the research committees in Sweden.

References
1. Sjöstedt A. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. Ann N Y Acad Sci 2007;1105:1–29.
2. Eliasson H, Bäck E. Tularemia in an emergent area in Sweden: an analysis of 234 cases in five years. Scand J Infect Dis 2007;39:880–889.
3. Larson MA, Fey PD, Hinrichs SH, Iwen PC. Francisella tularensis bacteria associated with feline tularemia in the United States. Emerg Infect Dis 2014;20:2068–2071.
4. Magee J, Steele RW, Kelly NR, Jacobs RF. Tularemia transmitted by a squirrel bite. Pediatr Infect Dis J 1989;8:123–125.
5. Abrahamian FM, Goldstein EJ. Microbiology of animal bite wound infections. Clin Microbiol Rev 2011;24:231–246.
6. Eliasson H, Lindbäck J, Nuorti JP, Arneborn M, Giesecke J et al. The 2000 tularemia outbreak: a case-control study of risk factors in disease-endemic and emergent areas, Sweden. Emerg Infect Dis 2002;8:956–960.
7. Brouqui P, Lascola B, Roux V, Raoult D. Chronic Bartonella quintana bacteremia in homeless patients. N Engl J Med 1999;340:184–189.
8. Fournier PE, Mainardi JL, Raoult D. Value of microimmunofluorescence for diagnosis and follow-up of Bartonella endocarditis. Clin Diag Lab Immunol 2002;9:795–801.
9. La Scola B, Raoult D. Serological cross-reactions between Bartonella quintana, Bartonella henselae, and Coxiella burnetii. J Clin Microbiol 1996;34:2270–2274.
10. Switaj K, Olzynska-Krowicka M, Zarnowska-Prymek H, Zaborowski P. Tularemia after tick exposure - typical presentation of rare disease misdiagnosed as atypical presentation of common diseases: a case report. Cases J 2009;2:7954.
11. Capellan J, Fong IW. Tularemia from a cat bite: case report and review of feline-associated tularemia. Clin Infect Dis 1993;16:472–475.
12. Tärnvik A, Chu MC. New approaches to diagnosis and therapy of tularemia. Ann N Y Acad Sci 2007;1105:378–404.
13. Penn RL. Francisella tularensis (tularemia). In: Mandell GL, Bennet JE, and Dolin R (editors). Principles and Practice of Infectious Diseases, 6th ed. New York: Churchill Livingstone; 2005. pp. 2674–2683.
14. Eliasson H, Olcén P, Jönsson S, Bäck E et al. Kinetics of the immune response associated with tularemia: comparison of an enzyme-linked immunosorbent assay, a tube agglutination test, and a novel whole-blood lymphocyte stimulation test. Clin Vaccine Immunol 2008;15:1238–1243.
15. Splettstoesser WD, Tomaso H, Al Dahouk S, Neubauer H, Schuff-Werner P. Diagnostic procedures in tularemia with special focus on molecular and immunological techniques. J Vet Med B Infect Dis Vet Public Health 2005;52:249–261.
16. Tärnvik A. Nature of protective immunity to Francisella tularensis. Rev Infect Dis 1989;11:440–451.
17. Ehrenborg C, Byström R, Hjelm E, Friman G, Holmberg M. High Bartonella spp. seroprevalence in a Swedish homeless population but no evidence of trench fever. Scand J Infect Dis 2008;40:208–215.
18. Ehrenborg C, Hagberg S, Alden J, Makitalo S, Myrdal G et al. First known case of Bartonella quintana endocarditis in Sweden. Scand J Infect Dis 2009;41:73–75.
19. Westling K, Farra A, Cars B, Ekblom AG, Sandstedt K et al. Cat bite wound infections: a prospective clinical and microbiological study at three emergency wards in Stockholm, Sweden. J Infect 2006;53:403–407.
20. Westling K, Farra A, Jorup C, Nordenberg A, Settergren B et al. Bartonella henselae antibodies after cat bite. Emerg Infect Dis 2008;14:1943–1944.
21. Fournier PE, Ndihokubwayo JB, Guidran J, Kelly PJ, Raoult D. Human pathogens in body and head lice. Emerg Infect Dis 2002;8:1515–1518.
22. Mcgill S, Wesslen L, Hjelm E, Holmberg M, Rolf C et al. Serological and epidemiological analysis of the prevalence of Bartonella spp. antibodies in Swedish elite orienteers 1992–93. Scand J Infect Dis 2001;33:423–428.
23. Mcgill S, Wesslen L, Hjelm E, Holmberg M, Auvinen MK et al. Bartonella spp. seroprevalence in healthy Swedish blood donors. Scand J Infect Dis 2005;37:723–730.
24. Hjelm E, Mcgill S, Blomqvist G. Prevalence of antibodies to Bartonella henselae, B. elizabethae and B. quintana in Swedish domestic cats. Scand J Infect Dis 2002;34:192–196.
25. da Costa PS, Bragitte ME, Greco DB. Antibodies to Rickettsia rickettsii, Rickettsia typhi, Coxiella burnetii, Bartonella henselae, Bartonella quintana, and Ehrlichia chaffeensis among healthy population in Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 2005;100:853–859.
26. Holmberg M, McGill S, Ehrenborg C, Wesslén L, Hjelm E et al. Evaluation of human seroreactivity to Bartonella species in Sweden. J Clin Microbiol 1999;37:1381–1384.
27. Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A et al. Bartonella (Rochalimaea) quintana endocarditis in three homeless men. N Engl J Med 1995;332:419–423.
28. Maurin M, Eb F, Etienne J, Raoult D. Serological cross-reactions between Bartonella and Chlamydia species: implications for diagnosis. J Clin Microbiol 1997;35:2283–2287.
29. Savitt AG, Mena-Taboada P, Monsalve G, Benach JL. Francisella tularensis infection-derived monoclonal antibodies provide detection, protection, and therapy. Clin Vaccine Immunol 2009;16:414–422.
30. Gilmore RD, Carpio AM, Kosoy MY, Gage KL. Molecular characterization of the sucB gene encoding the immunogenic dihydrolipoamide succinyltransferase protein of Bartonella vinsonii subsp. berkhoffii and Bartonella quintana. Infect Immun 2003;71:4818–4822.
31. Litwin CM, Johnson JM, Martins TB. The Bartonella henselae sucB gene encodes a dihydrolipoamide succinyltransferase protein reactive with sera from patients with cat-scratch disease. J Med Microbiol 2004;53:1221–1227.

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