Bartonella quintana in a 4000-Year-Old Human Tooth

Michel Drancourt,[1] Lam Tran-Hung,[1] Jean Courtin,[2] Henry de Lumley,[2] and Didier Raoult[1]

[1]Unité des Rickettsies, CNR UMR 6020, IFR 48, and [2]Laboratoire de Préhistoire du Muséum National d'Histoire Naturelle, CNRS UMR 6569, Faculté de Médecine, Université de la Méditerranée, Marseille, France

Bacteria of the genus *Bartonella* are transmitted by ectoparasites (lice, fleas, ticks) and have mammalian reservoirs in which they cause chronic, asymptomatic bacteremia. Humans are the reservoir of *B. quintana,* the louse-borne agent of trench fever. We detected DNA of *B. quintana* in the dental pulp of a person who died 4000 years ago.

Bacteria of the genus *Bartonella* are transmitted by ectoparasites—including lice, fleas, and ticks—and have mammalian reservoirs in which they cause chronic, asymptomatic bacteremia [1]. Humans are the only known reservoir of *B. quintana,* which may cause a variety of diseases—including asymptomatic chronic bacteremia and symptomatic trench fever, found in homeless people in North America and Europe [2–4]; blood-culture–negative endocarditis [5, 6]; chronic lymphadenopathy [7]; and bacillary angiomatosis, found in immunocompromised patients [8]. *B. quintana* is transmitted only by the body louse *Pediculus humanus corporis* [9]. Chronic, asymptomatic bacteremia caused by *B. quintana* is an exceptional situation in humans and provides a unique model for studying the age and coevolution of host-bacteria relationships. Trench fever was first reported during World War I, well before the isolation of the causative organism during the 1960s [8, 10]. Current studies have shown that up to 14% of homeless people with body lice have *B. quintana* bacteremia [11]. In ancient times, ectoparasitism was likely to be common, and infections with *B. quintana* might also have been prevalent. Because examination of dental pulp, which is similar to examination of a blood sample, might enable the detection of bacteremia, we tested for DNA of *B. quintana* in pulp from 12 teeth collected from human remains in southeastern France. In the present study, we demonstrate that *B. quintana* bacteremia has occurred in humans for ⩾4000 years.

MATERIALS AND METHODS

A total of 6 molars were obtained from 3 individuals whose remains were excavated from the archaeological site of Roaix, in southeastern France: 2 right maxillar molars from 1 individual, 3 right mandibular molars from 1 individual, and 1 left mandibular molar from 1 individual. In addition, 1 premolar and 3 molars were obtained from 1 individual, and 1 molar was obtained from each of 2 individuals whose remains were excavated from the archaeological site of Peyraoutes, also in southeastern France. Radiocarbon dating of portions of the skeletons indicated that the teeth were from humans who had died ~2100–2200 BC and ~2230–1950 BC, respectively, at the 2 sites [12]. Controls consisted of 17 teeth extracted during 2003 from patients with no evidence of past exposure to ectoparasites or *B. quintana* (figure 1*A*). Teeth were washed and radiographed in a laboratory located in a first building, as described elsewhere [13]. Extraction of DNA from the dental pulp was performed in a second building located ~500 m from the first building. Amplification and sequencing reactions were performed in a third building that was located ~300 m from each of the other 2 buildings. All experiments were done by 1 of the au-

Received 8 July 2004; accepted 11 August 2004; electronically published 10 January 2005.

Financial support: Unité des Rickettsies, Université de la Mediterranée.

Reprints or correspondence: Dr. Didier Raoult, Unité des Rickettsies, 27 Bd. Jean Moulin, 13385 Marseille Cedex 5, France (didier.raoult@medecine.univ-mrs.fr).

The Journal of Infectious Diseases 2005;191:607–11
© 2005 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2005/19104-0017$15.00

Downloaded from https://academic.oup.com/jid/article/191/4/607/939200 by guest on 18 April 2024

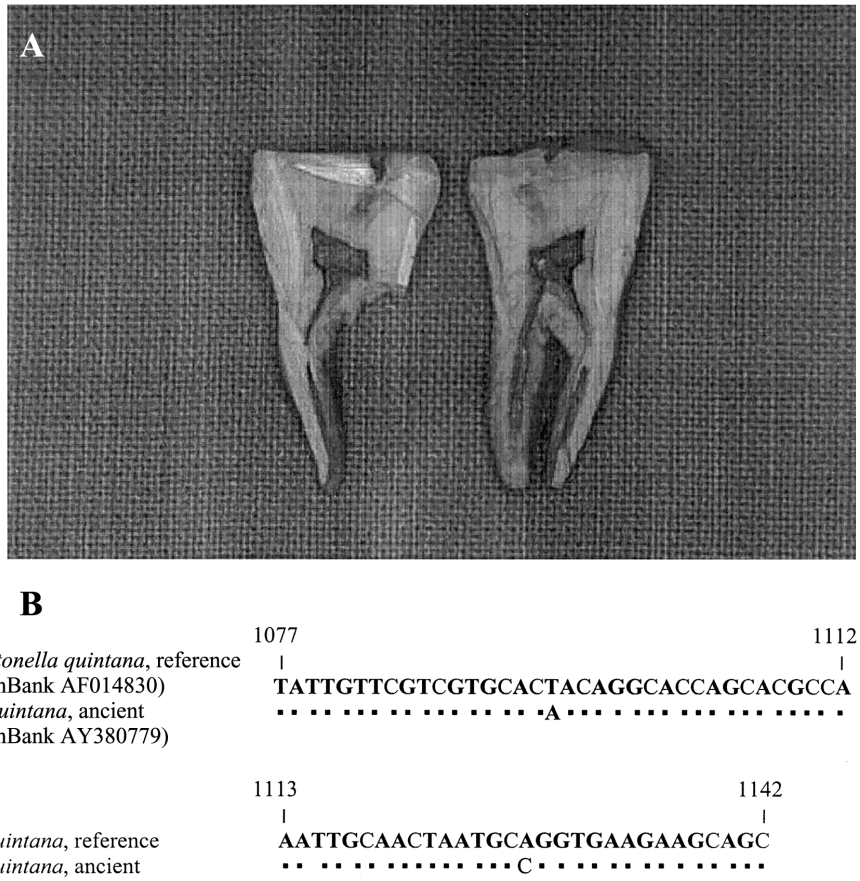

Figure 1. Recovery of 4000-year-old *Bartonella quintana* DNA. *A,* Opened premolar collected from a 4000-year-old individual, which yielded pulverulent dental pulp suitable for extraction of DNA. Amplification by polymerase chain reaction and sequencing reactions yielded 2 fragments of *B. quintana* DNA. *B,* 269-bp *Bartonella groEL* gene fragment sequenced from the same tooth, exhibiting 2 mutations not present in the homologous gene sequence from its closest relative, the modern *B. quintana* sequence in GenBank.

thors (L.T.-H.), who had no history of exposure to *B. quintana* or its DNA. New reagents and disposable materials were used for the experiments, as described elsewhere [14]. We attempted to amplify a 283-bp fragment of the *B. quintana* hemin-binding protein–E gene (EMBL accession number BX897700.1) from the dental pulp of all teeth by using a nested polymerase chain reaction (PCR), as described elsewhere [15], incorporating primer pairs designed for this experiment and never used before in our laboratory. The external primer pair was hbpEF1 (5′-GAGAGTGCTTCACCTAAATAG-3′) and hbpER1 (5′-CCACC-AATCTGTCCTCCAAA-3′); the internal primer pair was hbpEF2 (5′-GAGACGAGTATTAAAGTTTC-3′) and hbpER2 (5′-CTGAGGAACTATTACATCT-3′). Because this gene had never been amplified and studied in our laboratory, this genetic fragment was chosen so as to prevent the contamination of ancient DNA by modern amplicons. In brief, 2 μL of DNA extracted from the dental pulp were amplified in a 25-μL mixture containing 10-pmol/L solutions of each primer; 200 μL each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythimidine triphosphate (Invitrogen); 1.5 U/L *Taq* DNA polymerase (Invitrogen); and 2.5 μL of a 50 mmol/L solution of $MgCl_2$ in 1× *Taq* buffer. Amplification was performed with an initial denaturation at 94°C for 3 min, followed by 44 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 68°C for 90 s. The amplification was completed by keeping the reaction mixture at 68°C for 7 min. The amplicons obtained were sequenced directly, by use of internal primer pairs. We also attempted to amplify and sequence a 269-bp fragment of the *Bartonella groEL* gene from the dental pulp by using methods described elsewhere and an annealing temperature of 48°C [15].

RESULTS

No amplicons were obtained from the negative-control teeth, in either experiment. A genetic fragment 100% identical to that of the *B. quintana* hemin-binding protein–E gene was amplified and sequenced from the dental pulp of 1 molar from Peyraoutes. An amplicon of the *groEL* gene was generated from the

Downloaded from https://academic.oup.com/jid/article/191/4/607/939200 by guest on 18 April 2024

dental pulp of the same tooth (figure 1*B*), and, after it was cloned and sequenced, the 10 best similarity scores were obtained for the homologous gene in the modern *Bartonella* species. The amplicon had 64 of 66 base positions in common (97% similarity) with the modern *B. quintana groEL* gene (GenBank accession number AF014830) and 61 of 66 base positions in common (92% similarity) with the homologous gene in the modern *B. henselae* CAL-1 strain (GenBank accession number AF304020). The ancient sequence did, however, have 2 mutations (GenBank accession number AY380779) that have not previously been described in *B. quintana.*

DISCUSSION

In the present study, we have demonstrated for the first time the presence of *B. quintana* DNA in ancient human remains. We believe that our results were not influenced by contamination, because of the extensive precautions that we took: each experimental step was performed in a different building free of *B. quintana* and its DNA, we did not use a positive control in our PCRs, and we amplified a genetic fragment that had never previously been targeted in our laboratory (the "suicide PCR" protocol) [14]. We obtained no amplification products from any of our negative controls, dental pulp from the same tooth tested positive for *B. quintana* DNA in both tests, and the *groEL* sequence that we found has not been reported previously, further excluding the possibility of contamination by modern *B. quintana* DNA.

Specific bacterial DNA sequences were obtained from the dental pulp, suggesting that the individual studied had *B. quintana* bacteremia before death. We have shown elsewhere, in both experimental and clinical models, that dental pulp is equivalent to a small blood sample. Using *Coxiella burnetii* in a guinea pig model, we were able to recover specific bacterial DNA sequences [16] and viable microorganisms [17] from the dental pulp of experimentally infected animals. Specific viral DNA sequences were recovered from the dental pulp of HIV-infected patients [18, 19], and we recently demonstrated the presence of *B. quintana* DNA in the dental pulp of a bacteremic homeless patient who had been treated with an aminoglycoside and doxycycline a few weeks before removal of the tooth [20]. Furthermore, using dental pulp, we have been able to recover and sequence fragments of *Yersinia pestis* DNA in individuals suspected to have died of plague during the 6th–18th centuries, thus demonstrating that the 2 historical plague pandemics were likely caused by *Y. pestis* [13, 14, 21]. Also, we recently used dental pulp to demonstrate the presence of specific sequences of *B. henselae* DNA in feline remains from the 13th–16th centuries [22]. These data indicate that dental pulp is a versatile specimen that can be used when in a search for blood-borne pathogens in both living creatures and long-buried remains.

Our data indicate that humans were exposed to *B. quintana* ⩾4000 years ago. Trench fever, then, is one of the most ancient bacterial diseases in humans, as is tuberculosis, which has been detected in the remains of a person who died 5400 years ago in Egypt [23] (table 1). The present study is the first to report an ancient ectoparasite-borne bacterial disease in humans. Although the head louse most likely is one of the oldest permanent ectoparasites of humans, with infections caused by lice traced back 10,000 years [24], it is not a known vector for *B. quintana* [25]. There is little evidence for when lice first par-

Table 1. Molecular detection of bacterial DNA from human pathogens in ancient specimens.

Time period	Pathogen	Host	Specimen	Reference
17,000 BP	*Mycobacterium tuberculosis*	Bison	Bone	[29]
5400 BP	*M. tuberculosis*	Humans	Bone	[23]
4000 BP	*Bartonella quintana*	Humans	Dental pulp	Present study
3500 BP	*M. tuberculosis*	Humans	Lung	[30]
6th century AD	*Yersinia pestis*	Humans	Teeth	[31]
7th century AD	*M. leprae*	Humans	Bone	[32]
8th century AD	*Y. pestis*	Humans	Dental pulp	[21]
11th century AD	*M. tuberculosis*	Humans	Lung	[33]
12th century AD	*M. leprae*	Humans	Bone	[34]
13th century AD	*B. henselae*	Cats	Dental pulp	[22]
14th century AD	*Y. pestis*	Humans	Dental pulp	[21]
Medieval period	*M. tuberculosis*	Humans	Bone	[35–39]
	M. leprae	Humans	Bone	[40]
17th century AD	*Y. pestis*	Humans	Dental pulp	[13]
18th century AD	*Y. pestis*	Humans	Dental pulp	[13]
	Treponema pallidum	Humans	Bone	[41]
19th century AD	*Borrelia burgdorferi*	Ticks	Tick	[42]
		Rodents	Skin	[43]

Downloaded from https://academic.oup.com/jid/article/191/4/607/939200 by guest on 18 April 2024

asitized humans, but their eggs have been found in a prehistoric textile [26]; in textiles excavated at Masada, Israel, dated AD 66–73 [27]; and in the remains of fecal deposits of farmers in Viking Greenland, dated AD 986–1350 [28]. These few observations indicate that the body louse probably has parasitized humans—particularly those living in overcrowded conditions, such as in Masada—for millennia. These data may encourage scientists to use molecular techniques to explore ancient ectoparasites and human remains, in an attempt to research the history of ectoparasite-borne pathogens and to develop new insights into the coevolution of the host-bacteria relationship of *B. quintana* and humans.

References

1. Breitschwerdt EB, Kordick DL. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev **2000**; 13:428–38.
2. Foucault C, Barrau K, Brouqui P, Raoult D. *Bartonella quintana* bacteremia among homeless people. Clin Infect Dis **2002**; 35:684–9.
3. Raoult D, Foucault C, Brouqui P. Infections in the homeless. Lancet Infect Dis **2001**; 1:77–84.
4. Spach DH, Kanter AS, Dougherty MJ, et al. *Bartonella (Rochalimaea) quintana* bacteremia in inner-city patients with chronic alcoholism. N Engl J Med **1995**; 332:424–8.
5. Drancourt M, Mainardi JL, Brouqui P, et al. *Bartonella (Rochalimaea) quintana* endocarditis in three homeless men. N Engl J Med **1995**; 332: 419–23.
6. Fournier PE, Lelievre H, Eykyn SJ, et al. Epidemiologic and clinical characteristics of *Bartonella quintana* and *Bartonella henselae* endocarditis: a study of 48 patients. Medicine (Baltimore) **2001**; 80:245–51.
7. Raoult D, Drancourt M, Carta A, Gastaut JA. *Bartonella (Rochalimaea) quintana* isolation in patient with chronic adenopathy, lymphopenia, and a cat. Lancet **1994**; 343:977.
8. Maurin M, Roux V, Stein A, Ferrier F, Viraben R, Raoult D. Isolation and characterization by immunofluorescence, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, western blot, restriction fragment length polymorphism–PCR, 16S rRNA gene sequencing, and pulsed-field gel electrophoresis of *Rochalimaea quintana* from a patient with bacillary angiomatosis. J Clin Microbiol **1994**; 32:1166–71.
9. Ohl ME, Spach DH. *Bartonella quintana* and urban trench fever. Clin Infect Dis **2000**; 31:131–5.
10. Vinson JW. In vitro cultivation of the rickettsial agent of trench fever. Bull World Health Organ **1966**; 35:155–64.
11. Brouqui P, Lascola B, Roux V, Raoult D. Chronic *Bartonella quintana* bacteremia in homeless patients. N Engl J Med **1999**; 340:184–9.
12. Bard E, Rostek F, Menot-Combes G. Paleoclimate: a better radiocarbon clock. Science **2004**; 303:178–9.
13. Drancourt M, Aboudharam G, Signoli M, Dutour O, Raoult D. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. Proc Natl Acad Sci USA **1998**; 95:12637–40.
14. Raoult D, Aboudharam G, Crubezy E, Larrouy G, Ludes B, Drancourt M. Molecular identification by "suicide PCR" of *Yersinia pestis* as the agent of medieval black death. Proc Natl Acad Sci USA **2000**; 97: 12800–3.
15. Zeaiter Z, Fournier PE, Raoult D. Genomic variation of *Bartonella henselae* strains detected in lymph nodes of patients with cat scratch disease. J Clin Microbiol **2002**; 40:1023–30.
16. Aboudharam G, Lascola B, Raoult D, Drancourt M. Detection of *Coxiella burnetii* DNA in dental pulp during experimental bacteremia. Microb Pathog **2000**; 28:249–54.
17. Aboudharam G, Drancourt M, Raoult D. Culture of *C. burnetii* from the dental pulp of experimentally infected guinea pigs. Microb Pathog **2004**; 36:349–50.
18. Glick M, Trope M, Bagasra O, Pliskin ME. Human immunodeficiency virus infection of fibroblasts of dental pulp in seropositive patients. Oral Surg Oral Med Oral Pathol **1991**; 71:733–6.
19. Glick M, Trope M, Pliskin ME. Detection of HIV in the dental pulp of a patient with AIDS. J Am Dent Assoc **1989**; 119:649–50.
20. Aboudharam G, Foucault C, Fournier PE, Drancourt M, Brouqui P, Raoult D. Molecular detection of *Bartonella quintana* DNA in the dental pulp of a homeless patient. Eur J Clin Microbiol Infect Dis **2004**; 920-2.
21. Drancourt M, Roux V, La VD, et al. Genotyping, orientalis-like *Yersinia pestis,* and plague pandemics. Emerg Infect Dis **2004**; 10:1585–92.
22. La VD, Clavel B, Leptez S, Aboudharam G, Raoult D, Drancourt M. Molecular detection of *Bartonella henselae* DNA in the dental pulp of 800-year-old French cats. Clin Infect Dis **2004**; 39:1391–4.
23. Crubezy E, Ludes B, Poveda JD, Clayton J, Crouau-Roy B, Montagnon D. Identification of *Mycobacterium* DNA in an Egyptian Pott's disease of 5,400 years old. C R Acad Sci III **1998**; 321:941–51.
24. Araujo A, Ferreira LF, Guidon N, Maues Da Serra FN, Reinhard KJ, Dittmar K. Ten thousand years of head lice infection. Parasitol Today **2000**; 16:269.
25. Fournier PE, Ndihokubwayo JB, Guidran J, Kelly PJ, Raoult D. Human pathogens in body and head lice. Emerg Infect Dis **2002**; 8:1515–8.
26. Hundt HJ. Vorgeschichtliche Gewebe aus dem Hallstaetter Salzberg. Jahrbuch des Roemisch-Germanischen Zentralmuseums Mainz **1960**; 7:126–41.
27. Mumcuoglu KY, Zias J, Tarshis M, Lavi M, Stiebel GD. Body louse remains found in textiles excavated at Masada, Israel. J Med Entomol **2003**; 40:585–7.
28. Sadler JP. Records of ectoparasites on humans and sheep from Viking-age deposits in the former western settlement of Greenland. J Med Entomol **1990**; 27:628–31.
29. Rothschild BM, Martin LD, Lev G, et al. *Mycobacterium tuberculosis* complex DNA from an extinct bison dated 17,000 years before the present. Clin Infect Dis **2001**; 33:305–11.
30. Nerlich AG, Haas CJ, Zink A, Szeimies U, Hagedorn HG. Molecular evidence for tuberculosis in an ancient Egyptian mummy. Lancet **1997**; 350:1404.
31. Wiechmann I, Grupe G. Detection of *Yersinia pestis* DNA in two early medieval skeletal finds from Aschheim (Upper Bavaria, 6th century A.D.). Am J Phys Anthropol **2005**; 126:48-55.
32. Rafi A, Spigelman M, Stanford J, Lemma E, Donoghue H, Zias J. *Mycobacterium leprae* DNA from ancient bone detected by PCR. Lancet **1994**; 343:1360–1.
33. Salo WL, Aufderheide AC, Buikstra J, Holcomb TA. Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. Proc Natl Acad Sci USA **1994**; 91:2091–4.
34. Montiel R, Garcia C, Canadas MP, Isidro A, Guijo JM, Malgosa A. DNA sequences of *Mycobacterium leprae* recovered from ancient bones. FEMS Microbiol Lett **2003**; 226:413–4.
35. Konomi N, Lebwohl E, Mowbray K, Tattersall I, Zhang D. Detection of mycobacterial DNA in Andean mummies. J Clin Microbiol **2002**; 40:4738–40.
36. Mays S, Fysh E, Taylor GM. Investigation of the link between visceral surface rib lesions and tuberculosis in a medieval skeletal series from England using ancient DNA. Am J Phys Anthropol **2002**; 119:27–36.
37. Spigelman M, Lemma E. The use of the polymerase chain reaction to detect *Mycobacterium tuberculosis* in ancient skeletons. Int J Osteoarchaeol **1993**; 3:143.
38. Taylor GM, Crossey M, Saldanha JA, Waldron T. Detection of *Mycobacterium tuberculosis* bacterial DNA in medieval human skeletal remains using polymerase chain reaction. J Archaeol Sci **1996**; 23:789–98.
39. Taylor GM, Goyal M, Legge AJ, Shaw RJ, Young D. Genotypic analysis of *Mycobacterium tuberculosis* from medieval human remains. Microbiology **1999**; 145:899–904.

Downloaded from https://academic.oup.com/jid/article/191/4/607/939200 by guest on 18 April 2024

40. Haas CJ, Zink A, Palfi G, Szeimies U, Nerlich AG. Detection of leprosy in ancient human skeletal remains by molecular identification of *Mycobacterium leprae.* Am J Clin Pathol **2000**; 114:428–36.
41. Kolman CJ, Centurion-Lara A, Lukehart SA, Owsley DW, Tuross N. Identification of *Treponema pallidum* subspecies pallidum in a 200-year-old skeletal specimen. J Infect Dis **1999**; 180:2060–3.
42. Matuschka FR, Ohlenbusch A, Eiffert H, Richter D, Spielman A. Characteristics of Lyme disease spirochetes in archived European ticks. J Infect Dis **1996**; 174:424–6.
43. Marshall WF 3rd, Telford SR 3rd, Rys PN, et al. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Peromyscus leucopus.* J Infect Dis **1994**; 170:1027–32.

Downloaded from https://academic.oup.com/jid/article/191/4/607/939200 by guest on 18 April 2024