2. Karam NE, Roger L, Hankins LL, Reveille JD. Rheumatoid nodulosis of the meninges. J Rheumatol. 1994;21:1960–3.
3. Kim RC, Collins GH. The neuropathology of rheumatoid disease. Hum Pathol. 1981;12:5–15.
4. Han XY, Pham AS, Tarrand JJ, Sood PK, Luthra R. Rapid and accurate identification of mycobacteria by sequencing hypervariable regions of the 16S ribosomal RNA gene. Am J Clin Pathol. 2002;118:796–801.
5. Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev. 1996;9:18–33.

Address for correspondence: X. Y. Han, Department of Laboratory Medicine, Unit 84, University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, Texas, USA; fax: 713-792-0936; email: xhan@mdanderson.org

In Response: In response to our report on a case of rapidly progressive dementia (1,2), Dr. Han argues that *Mycobacterium neoaurum* was "more likely a contaminant than the cause" and that the actual cause of death was most likely rheumatoid pachymeningitis. Dr. Han bases his argument on the absence of positive acid-fast stains or mycobacterial cultures and his assessments that the identification of *M. neoaurum* DNA was due to contamination and that the pathologic findings represented rheumatoid nodules.

The inability to stain or culture an organism in this case is not unusual, as paucibacillary mycobacterial infections, such as tuberculous lymphadenitis and leprosy, are common (3,4). Though the possibility is not inconceivable, environmental contamination is unlikely, because tissue samples were positive with *M. neoaurum*–specific primers, whereas controls containing identical reagents but no tissue were not.

Dr. Han expresses a valid concern that rheumatoid pachymeningitis was not given due consideration. Rheumatoid pachymeningitis is a rare complication of rheumatoid arthritis, in which patients may exhibit headache, cranial neuropathies, focal deficits, seizures, or cognitive dysfunction (5,6). Rheumatoid pachymeningitis usually, but not exclusively, occurs in patients with long-standing rheumatoid arthritis characterized by erosive disease and extra-articular manifestations, although the systemic disease may be quiescent when neurologic complications arise. Cerebrospinal fluid analysis is generally nonspecific. Magnetic resonance imaging may show prominent meningeal enhancement. Pathologic features may include vasculitis, rheumatoid nodules, and meningeal inflammation, with the latter 2 features being most common (5). The dura may demonstrate inflammation with fibrinoid necrosis (6). We reviewed the pathologic specimens of this case and confirmed the presence of abundant giant cells, endarteritis proliferans, and, most notably, extensive caseation necrosis typical of mycobacterial infection. We found no evidence of rheumatoid nodules, dural inflammation, or fibrinoid necrosis.

Though this case does not satisfy Koch postulates, neither do most novel infectious diseases. Substantial international efforts were required to satisfy the postulates in the case of SARS (7). In this case, the identification of DNA from a "rare environmental mycobacterium" in a patient with overwhelming pathologic evidence of mycobacterial infection provides strong, though not foolproof, evidence of a possible causal role.

George A. Heckman,*
Cynthia Hawkins,† Andrew
Morris,‡ Lori L. Burrows,†
and Catherine Bergeron§
*Chedoke Campus and Freeport Health Centre, Kitchener, Ontario, Canada; †University of Toronto, Toronto, Ontario, Canada; ‡McMaster University, Hamilton, Ontario, Canada; §Toronto Western Hospital, Toronto, Ontario, Canada

References

1. Han XY. *Mycobacterium neoaurum* contamination. Emerg Infect Dis. 2005;11:1316–7.
2. Heckman GA, Hawkins C, Morris A, Burrows LL, Bergeron C. Rapidly progressive dementia due to *Mycobacterium neoaurum* meningoencephalitis. Emerg Infect Dis. 2004;10:924–7.
3. Chao SS, Loh KS, Tan KK, Chong SM. Tuberculous and nontuberculous cervical lymphadenitis: a clinical review. Otolaryngol Head Neck Surg. 2002;126:176–9.
4. Ustianowski AP, Lockwood DNJ. Leprosy: current diagnostic and treatment approaches. Cur Opin Infect Dis. 2003;16:421–7.
5. Kato T, Hoshi K, Sekijima Y, Matsuda M, Hashimoto T, Otani M, et al. Rheumatoid meningitis: an autopsy report and review of the literature. Clin Rheumatol. 2003;22:475–80.
6. Tan HJ, Raymond AA, Phadke PP, Rozman Z. Rheumatoid pachymeningitis. Singapore Med J. 2004;45:337–9.
7. Osterhaus ADME, Fouchier RAM, Kuiken T. The aetiology of SARS: Koch's postulates fulfilled. Philos Trans R Soc Lond B Biol Sci. 2004;359:1081–2.

Address for correspondence: George A. Heckman, Department of Medicine, Hamilton Health Sciences, Chedoke Campus and Freeport Health Centre, 3570 King Street East, Kitchener, Ontario, Canada, N2C 2W1; fax: 519-894-8326; email: heck0@sympatico.ca

Yersinia pestis Genotyping

To the Editor: Drancourt et al. (1) report the development of an original genotyping system for *Yersinia pestis* based on intergenic spacer sequencing. However, the approach appears to rely upon the characterization of polymorphisms due to tandem repeat variation. Eight spacers are used in the report, 7 of which contain tandem repeats, and the sequence variability used to produce the typing data and

the strain clustering result from variation in the number of tandem repeats (and incorrect data analysis produces a dendrogram with 34 branches from only 19 different isolate types). Three of the spacers and associated polymorphisms were previously reported. Spacers YP3 and YP5 are, respectively, ms38 and ms56 (2); spacer YP10 is M61 (3). YP3 is later used to investigate ancient DNA samples, and 3 amplification products are described in detail. The sequences are compared to modern sequences by BLAST analysis, which is not relevant for tandem repeats. Instead, the Figure shows how internal variation within the array can be coded to facilitate interpretation. In this collection, Orientalis strains are "abcdeeef," whereas Antiqua strains from Africa are "abcdeef." All these different codes can be deduced one from the other by simple duplication and deletion events, with no need to invoke point mutations. The codes for all 3 ancient samples are identical to the Orientalis code "abcdeeef."

In conclusion, the data presented by Drancourt et al. do not appear to support their claim. They did not invent a new genotyping method but used the well-known multiple locus variable analysis (MLVA) number of tandem repeats approach. The finding that the "genotype Orientalis was involved in all three pandemics" is not valid since the Orientalis type is defined by a biochemical assay, resulting in all known Orientalis strains from a 93-bp glycerol-3-phosphate dehydrogenase microdeletion (4,5), which was not investigated here.

Gilles Vergnaud*

*Centre d'Etudes du Bouchet, Vert le Petit, France

References

1. Drancourt M, Roux V, Dang LV, Tran-Hung L, Castex D, Chenal-Francisque V, et al. Genotyping, Orientalis-like *Yersinia pestis*, and plague pandemics. Emerg Infect Dis. 2004;10:1585–92.
2. Le Flèche P, Hauck Y, Onteniente L, Prieur A, Denoeud F, Ramisse V, et al. A tandem repeats database for bacterial genomes: application to the genotyping of *Yersinia pestis* and *Bacillus anthracis*. BMC Microbiol. 2001;1:2.
3. Klevytska AM, Price LB, Schupp JM, Worsham PL, Wong J, Keim P. Identification and characterization of variable-number tandem repeats in the *Yersinia pestis* genome. J Clin Microbiol. 2001;39:3179–85.
4. Motin VL, Georgescu AM, Elliott JM, Hu P, Worsham PL, Ott LL, et al. Genetic variability of *Yersinia pestis* isolates as predicted by PCR-based IS100 genotyping and analysis of structural genes encoding glycerol-3-phosphate dehydrogenase (glpD). J Bacteriol. 2002;184:1019–27.
5. Pourcel C, Andre-Mazeaud F, Neubauer H, Ramisse F, Vergnaud G. Tandem repeats analysis for the high resolution phylogenetic analysis of *Yersinia pestis*. BMC Microbiol. 2004;4:22.

Address for correspondence: Gilles Vergnaud, Department of Analytical Microbiology, Centre d'Etudes du Bouchet 91710, Vert le Petit, France; fax: 33-1-69-15-66-78; email: gilles.vergnaud@igmors.u-psud.fr

In response: We thank Dr. Vergnaud for his response (1). Since the time of our publication (2), 2 articles related to our paper were either submitted or published. One (3) reported identification of *Yersinia pestis*–specific genes in teeth from patients who died during the Justinian plague; another proposed identification of *Y. pestis* strains by using variable numbers of tandem repeats analysis (VNTR) (4). The authors concluded that isolates could easily be compared in their database by using 7 markers. As opposed to work with cultures where ample, high-quality DNA template is available, successful amplifications with 7 different primer sets cannot be achieved by using DNA extracted from ancient teeth (5). By comparing genome sequences, we evaluated short intergenic spacers that were more divergent. Divergences included mutations, deletions, and duplications (VNTR). Phylogenetically, an entire repeat unit has the same weight as that of a single nucleotide polymorphism. By sequencing, we have identified all events (single nucleotide polymorphism and VNTR). Sequencing is more versatile for use in strain identification (5),


```
A
    'dictionary'
a :  --G---G--------A
b :  --G---G-T-------
c :  -----T-T----G---
d :  ------------G---
e :  ACCAGCTCCAACAATT
f :  -T---T-T--------
g :  -----T----------
h :  -----T------G---

B
    internal coding
Medievalis        abceef
Antiqua           abcdeef
Antiqua (Asia)    abcdceef
Orientalis        abcdeeef
#202 (Justinien)  abcdeeef
#283              abcdeeef
#292              abcdeeef
Ypseu             abcdgdhhhef
Microtus          abcdceef
```

Figure. A) sequence-to-code correspondence (1 letter per 16-bp repeat unit). Differences from repeat unit "e" are shown. B) Tandem repeat arrays were coded accordingly. All sequences were obtained from Genbank (Ypseu: *Yersinia pseudotuberculosis* IP32953; Microtus: "*Y. microtus*" Chinese strain #91001).
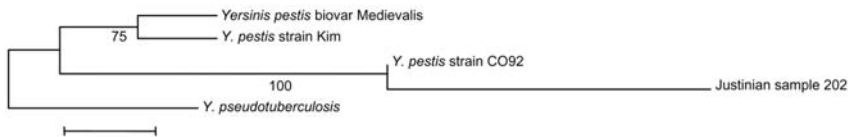
Figure: Unrooted tree showing the phylogenetic relationships between the sequence obtained from the YP3 spacer from Justinian sample 202 and that from the genomes of *Yersinia pestis* strain CO92 (GenBank accession no. AJ414159), *Y. pestis* biovar Medievalis (AE017139), *Y. pestis* strain Kim (AE013993), and *Y. pseudotuberculosis* (BX936398). DNA sequences were aligned by using the ClustalW software, version 1.81 (2). Deletions were considered single events. A distance matrix was constructed by using the Kimura-2 parameter, and the phylogenetic tree was inferred by using the neighbor-joining method in the Mega2 software package. The scale bar represents a 0.5% nucleotide sequence divergence. Bootstrap values are indicated at the nodes (2).

allows distinction at the species level, and can be applied directly on clinical and forensic samples.

The discovery of a unique sequence is critical to authenticate results in such controversial areas as paleomicrobiology (5). Fortunately, we have identified a unique sequence that contains several mutations. These mutations do not exclude this strain from being *Y. pestis* (see Figure). Additionally, we doubt that our conclusions would have been accepted had we simply used the VNTR, demonstrating only an amplicon of the right size on a gel.

In conclusion, our results have been validated by others. The sequence is original and, therefore, authentic. Dr. Vergnaud agrees that the results we presented did represent a sequence associated with the Orientalis biovar. This finding may end the controversy.

Didier Raoult,* Michel Drancourt,* Pierre Edouard Fournier,* and Hiro Ogata*

*Centre National de Reference, Marseille, France

References

1. Vergnaud G. *Yersinia pestis* genotyping. Emerg Infect Dis. 2005;11:1317–8.
2. Drancourt M, Roux V, Dang LV, Tran-Hung L, Castex D, Chenal-Francisque V, et al. Genotyping, Orientalis-like *Yersinia pestis*, and plague pandemics. Emerg Infect Dis. 2004;10:1585–92.
3. Weichmann I, Grupe G. Detection of *Yersinia pestis* DNA in two early medieval skeletal finds from Aschheim (Upper Bavaria, 6th century AD). Am J Phys Anthropol. 2005;126:48–55.
4. Pourcel C, Andre-Mazeaud F, Neubauer H, Ramisse F, Vergnaud G. Tandem repeats analysis for the high resolution phylogenetic analysis of *Yersinia pestis*. BMC Microbiol. 2004;4:22.
5. Drancourt M, Raoult D. Paleomicrobiology: current issues and perspectives. Nat Rev Microbiol. 2005;3:23–35.

Address for correspondence: Didier Raoult, Unité des Rickettsies, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France; fax: 33-4-91-38-77-72; email: didier.raoult@medecine.univ-mrs.fr

Pandemic *Vibrio parahaemolyticus* O3:K6, Europe

To the Editor: *Vibrio parahaemolyticus* is a halophilic member of the genus *Vibrio* that inhabits temperate and tropical marine environments worldwide. Strains that produce the thermostable direct hemolysin or the thermostable direct hemolysin-related hemolysin, which are encoded by *tdh* and *trh* genes, respectively, are considered pathogenic. While almost all clinical strains have these virulence factors, these strains represent <1% of all environmental strains.

Recently, *V. parahaemolyticus* infections have increased globally; they are usually associated with eating raw or undercooked seafood. *V. parahaemolyticus* is the leading cause of seafood-associated bacterial gastroenteritis in the United States (1) and causes approximately half of the foodborne outbreaks in some Asian countries (2). In 2001, the Scientific Committee on Veterinary Measures Relating to Public Health of the European Commission concluded that *V. parahaemolyticus* outbreaks are rarely reported in Europe (3). Because the risk of *V. parahaemolyticus* infection is extremely low in Europe, the organism has been excluded from the European Network for Epidemiologic Surveillance and Control of Communicable Diseases and from Microbiologic Surveillance System for Infectious Gastroenteritis. *V. parahaemolyticus* is also excluded from the European applicable microbiologic requirements for shellfish-harvesting areas and ready-to-eat seafood.

However, data obtained after an exhaustive review of clinical journals published in Spain and from unreported cases of *V. parahaemolyticus* infections identified at Spanish hospitals have shown that *V. parahaemolyticus* infections in Spain are more common than previously assumed. This organism was isolated from patients with gastroenteritis in Barcelona (1986, 1987, and 1999), Zaragoza (1993), and Madrid (1998 and 2000). In Galicia (northwestern Spain) alone, where most Spanish shellfish are produced, 84 cases of *V. parahaemolyticus* infection were identified retrospectively from hospital records from 1997 to 2000. A single outbreak of 64 cases in 1999 was associated with oyster consumption (4). Most Spanish clinical isolates were serotype O4:K11, and pulsed-field gel elec-