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 negative.

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.

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**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

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References


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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 “abcdeef,” whereas Antiqua strains from Africa are “abcede.” 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 “abcdeef.”

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.

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References

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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

<table>
<thead>
<tr>
<th>‘dictionary’</th>
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<tbody>
<tr>
<td>a : G--G--G-------A</td>
</tr>
<tr>
<td>b : G--G--T--------</td>
</tr>
<tr>
<td>c : ------T-T-------G-</td>
</tr>
<tr>
<td>d : -----------------</td>
</tr>
<tr>
<td>e : ACCAGCTTCAAACG</td>
</tr>
<tr>
<td>f : -T-----T--------</td>
</tr>
<tr>
<td>g : ------T----------</td>
</tr>
<tr>
<td>h : ------T----------</td>
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</tbody>
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B

<table>
<thead>
<tr>
<th>Internal coding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medievalis : abceef</td>
</tr>
<tr>
<td>Antiqua : abceef</td>
</tr>
<tr>
<td>Antiqua (Asia) : abceef</td>
</tr>
<tr>
<td>Orientalis : abceef</td>
</tr>
<tr>
<td>#202 (Justinien) : abceef</td>
</tr>
<tr>
<td>#283 : abceef</td>
</tr>
<tr>
<td>#292 : abceef</td>
</tr>
<tr>
<td>Ypseu : abcdggghheef</td>
</tr>
<tr>
<td>Microtus : abcdceef</td>
</tr>
</tbody>
</table>

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).
End the controversy.

Orientalis biovar. This finding may result 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 allowed 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.

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

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**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-