Obtained sequences were aligned and analyzed by using Bioedit software (Ibis Biosciences, Carlsbad, CA, USA) and ClustalW version 2.0.10 (www.ebi.ac.uk/clustalw). DAMBE (http://dambe.bio.uottawa.ca/dambe.asp) and MEGA (www.megasoftware.net) software were used to obtain similarity percentages among analyzed sequences. To characterize Rickettsia spp., we used nucleotide sequence identity to reference strains (10).

Convalescent-phase serum was positive for *R. conorii*; IgG titer was 64. Sequence analysis of purified PCR products obtained from the eschar identified the isolate as *R. massiliae*. With respect to the reference strain *R. massiliae*, pairwise nucleotide sequence identity was 99% for the *gltA* gene (GenBank accession no. JN043507), 99% for the *ompA* gene (accession no. JQ480842), and 97% for the *ompB* gene (accession no. AF123714). Phylogenetic analysis (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/5/12-1169-Techapp1.pdf) also confirmed the identity of the *Rickettsia* species.

Considering the diagnosis of *R. massiliae* infection and the patient who had acute vision loss (5), this patient was called back for a fundus examination, which showed no changes. At the time of this visit, a small area of alopecia at the eschar site was observed (Figure). Unfortunately, the tick had been discarded and was not available for genus and species identification.

The presence of *R. massiliae* in Italy demonstrates that this Rickettsia species can cause scalp eschar and neck lymphadenopathy. Further studies are needed to complete the list of microorganisms that can cause this condition and to understand if they can be associated with minor findings (e.g., alopecia, painful eschar, high fever).

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References

1. Angelakis E, Pulcini C, Waton J, Imbert P, Socolovschi C, Edouard S, et al. Scalp eschar and neck lymphadenopathy caused by *Bartonella henselae* after tick bite. Clin Infect Dis. 2010;50:549–51. http://dx.doi.org/10.1086/650172
2. Parola P, Rovery C, Rolain JM, Brouqui P, Davoust B, Raoult D. *Rickettsia slovaca* and *R. raoultii* in tick-borne rickettsioses. Emerg Infect Dis. 2009;15:1105–8. http://dx.doi.org/10.3201/eid1507.081449
3. García-García JC, Portillo A, Nunez MJ, Santibanez S, Castro B, Oteo JA. A patient from Argentina infected with *Rickettsia massiliae*. Am J Trop Med Hyg. 2010;82:691–2. http://dx.doi.org/10.4269/ajtmh.2010.09-0662
4. Vitale G, Mansuelo S, Rolain JM, Raoult D. *Rickettsia massiliae* human isolation. Emerg Infect Dis. 2006;12:174–5. http://dx.doi.org/10.3201/eid1201.050850
5. Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS Negl Trop Dis. 2008;2:e338. http://dx.doi.org/10.1371/journal.pntd.0000338
6. Tzianabos T, Anderson BE, McDade JE. Detection of *Rickettsia rickettsii* DNA in clinical specimens by using polymerase chain reaction technology. J Clin Microbiol. 1989;27:2866–8.
7. Oteo JA, Portillo A, Santibanez S, Blanco JR, Perez-Martinez L, Ibarra V. Cluster of cases of human *Rickettsia felis* infection from southern Europe (Spain) diagnosed by PCR. J Clin Microbiol. 2006;44:2669–71. http://dx.doi.org/10.1128/JCM.00366-06
8. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol. 1991;173:1576–89.
9. Choi YJ, Jang WJ, Ryu JS, Lee SH, Park KH, Paik HS, et al. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg Infect Dis. 2005;11:237–44. http://dx.doi.org/10.3201/eid1102.040603
10. Zhu Y, Fournier PE, Eremeeva M, Raoult D. Proposal to create subspecies of *Rickettsia conorii* based on multilocus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol. 2005;5:11. http://dx.doi.org/10.1186/1471-2180-5-11

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Mycobacterium tuberculosis Complex in Remains of 18th–19th Century Slaves, Brazil

To the Editor: Nineteenth century Rio de Janeiro, Brazil, was marked by increased illness and deaths from tuberculosis (TB). By the twentieth century, it was still believed that most TB cases in the Americas originated from Europe; the "virgin soil" hypothesis for African (1) and Amerindian populations was accepted. However, modern and archeological DNA evidence confirms the wide distribution of *Mycobacterium tuberculosis* complex (MTC) and TB in the Old and New Worlds.

Rio de Janeiro was a main entry port for millions of Africans captured for the slave trade. Pretos Novos (New Blacks) Cemetery (PNC; 1769–1830) was created in Rio de Janeiro as a burial ground for the many slaves who died at market. Comingled bone

fragments (≈5,000) from ≈30 persons were recovered at PNC; most bones were broken and had been exposed to fire (*2,3*). Bioanthropological analysis determined most of the bones were from men 18–25 years of age (*2*); none had lesions consistent with TB.

Femurs from 16 persons were surveyed for *M. tuberculosis* ancient DNA (aDNA). The thick shafts of femur offered a preserved condition for molecular analysis, and the bones could be individualized, avoiding duplication of samples. Paleogenetic investigation guidelines were followed. Sample preparation, aDNA extraction, and PCR were performed at the Paleogenetics Unit (Oswaldo Cruz Foundation, Rio de Janeiro) an isolated environment exclusively dedicated to aDNA research.

Before removal of the bone surface, samples were decontaminated by ultraviolet light (15 min/all sides), frozen in liquid nitrogen, and subjected to manual trituration. Bone powder (≈200 mg) was then incubated with digestion buffer (56°C, 48–72 h) as described (*4*). aDNA hybridizations with MTC probes were conducted as described (*4*). By using 2 segments of mitochondrial DNA (mtDNA) hypervariable segment I (HVS-I) target, we determined the ancestry of the humans from whom the bones were derived (*4*). To control for recent contamination, we compared the HVS-I sequences with those in GenBank and also in a database for the laboratory staff.

Using the hybridization assay with insertion sequence (IS) 6110 target, we detected MTC aDNA in bones from 4/16 persons (samples PN1, PN8, PN13, PN15); 3 of these samples (PN1, PN8, PN13) were also positive for IS1081 target, confirming MTC infection (Table). HVS-I target was retrieved from 3 samples (PN6, PN14, PN15), which enabled determination of the human mtDNA haplotypes (L3e2, L3d1, L1c2, respectively) (Table). The haplotypes showed that the 3 persons were of African descent (GenBank accession nos. JQ639893–Q639895). Our findings are consistent with those from studies based on current African populations, which show that haplotype L1c is restricted to central Africa (*5*) and L3d and L3e are most frequently found in western and central Africa, respectively (*6*).

Historic data (*3*) showed that 95% of persons buried in PNC were New Blacks, meaning they were born in Africa and died just after arriving in America. Our mtDNA results confirm historic and genetic records that indicate a large percentage of persons brought to Brazil as slaves originated from western–central and western Africa. This makes the PNC samples unique for the paleogenetic purpose of this investigation.

The endemicity of TB in Rio de Janeiro during the colonial period was confirmed by Jaeger and colleagues, who demonstrated MTC infection in 56.6% of persons with European ancestry buried at Nossa Senhora do Carmo Church (*4*). The difference in the frequency of MTC found in the remains of slaves buried in PNC and of Europeans buried at Nossa Senhora do Carmo Church may be explained by the types of samples analyzed and the epidemiologic conditions of both groups. The cremation of corpses at PNC may also partly explain the difference. The finding of MTC aDNA in the remains of 25% of persons buried at PNC could be an underestimation of infection.

Our finding that some of the slaves buried in PNC had TB infection

Table. Results of genetic analyses of *Mycobacterium tuberculosis* complex hybridization and human mtDNA haplotypes from human bone samples collected from Pretos Novos Cemetery, Rio de Janeiro, Brazil*

	TB hybridization		Nucleotide position of mtDNA hypervariable segment I†												mtDNA
Sample	IS6110	IS1081	129	145	148	172	187	189	213	223	278	311	319	320	haplotype
CRS	NA	NA	G	G	C	T	C	C	G	C	C	T	G	C	H
PN1	+	+													
PN2	–	–													
PN3	–	–													
PN4	–	–													
PN5	–	–													
PN6	–	–	G	G	C	T	C	T	G	T	C	C	G	T	L3e2
PN7	–	–													
PN8	+	+													
PN9	–	–													
PN10	–	–													
PN11	–	–													
PN12	–	–													
PN13	+	+													
PN14	–	–	G	G	C	T	C	T	G	T	C	T	A	C	L3d1
PN15	+	–	A	A	C	T	T	C	A	T					L1c2
PN16	–	–													

*The cemetery was used as a burial ground for African slaves who died in slave markets during 1769–1830. Blank cells mean target could not be retrieved. mtDNA, mitochondrial DNA; TB, tuberculosis; IS, insertion sequence; CRS, Cambridge Reference Sequence (accession no. AB055387); NA, not applicable; PN, bone samples from humans buried in Pretos Novos Cemetery; Abs, absence of nucleotide.
†The prefix 16 has been omitted from the nucleotide numbers.

when they arrived in Brazil is in agreement with previous findings of the differential distribution of TB and with a tuberculin survey on the African continent, supporting the hypothesis of native African TB (7,8). Therefore, the hypothesis of Africa as virgin soil for TB (1,9) cannot be easily supported. The incidence of TB among the slaves/ Blacks in Rio de Janeiro was less than expected given their social and sanitary conditions (10), especially in a TB-endemic situation (4). Previous exposure to MTC might explain their apparent relative resistance.

Other evidence showing African contact with Europeans before the sixteenth century, supports the existence of TB in Africa (8), and TB was prevalent in urbanized centers along coastal areas of western Africa (7,8). Although some of those cases were probably the result of European contact, it is not possible to exclude that some were caused by TB native to Africa. We can affirm that persons buried in PNC, who were transported to Brazil as slaves from Africa, brought TB infection with them; whether the infection was caused by European TB endemic to Africa or by TB native to Africa is not known.

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References

1. Cummins SL. "Virgin soil"–and after: a working conception of tuberculosis in children, adolescents, and aborigines. BMJ. 1929;2:39–41. http://dx.doi.org/10.1136/bmj.2.3575.39
2. Machado LC. Sítio cemitério dos Pretos Novos: análise biocultural. In: Dias O, Carvalho E, Zimmermann M, editors. Estudos contemporâneos de arqueologia. Palmas (Brazil): Fundação Universidade do Tocantins Neurosci, Instituto de Arqueologia Brasileira; 2006.
3. Pereira JCMS. À flor da terra: o cemitério dos Pretos Novos no Rio de Janeiro. Rio de Janeiro: Garamond; 2007.
4. Jaeger LH, Leles D, Lima VD, Silva LD, Neto OD, Iniguez AM. *Mycobacterium tuberculosis* complex detection in human remains: tuberculosis spread since the 17th century in Rio de Janeiro, Brazil. Infect Genet Evol. 2012;12:642–8. http://dx.doi.org/10.1016/j.meegid.2011.08.021
5. Harich N, Costa MD, Fernandes V, Kandil M, Pereira JB, Silva NM, et al. The trans-Saharan slave trade–clues from interpolation analyses and high-resolution characterization of mitochondrial DNA lineages. BMC Evol Biol. 2010;10:138. http://dx.doi.org/10.1186/1471-2148-10-138
6. Salas A, Richards M, De la Fe T, Lareu MV, Sobrino B, Sanchez-Diz P, et al. The making of the African mtDNA landscape. Am J Hum Genet. 2002;71:1082–111. http://dx.doi.org/10.1086/344348
7. Calmette A. Enquete sur l'epideiyiiologie de la tuberculose dans les colonies Françaises. Ann Inst Pasteur (Paris). 1912;7:497–514.
8. Daniel TM. The early history of tuberculosis in central East Africa: insights from the clinical records of the first twenty years of Mengo Hospital and review of relevant literature. Int J Tuberc Lung Dis. 1998;2:784–90.
9. Stead WW. Tuberculosis in Africa. Int J Tuberc Lung Dis. 1998;2:791–2.
10. Sigaud JFX. Do clima e das doenças do Brasil, estatística médica deste império. Rio de Janeiro: Editora Fiocruz. 2009.

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Treatment of Listeriosis in First Trimester of Pregnancy

To the Editor: Foodborne infections with *Listeria monocytogenes* continue to be dangerous and disruptive. A 2011 outbreak in the United States, linked to cantaloupes, affected 147 persons; 33 persons died, and 1 pregnant woman experienced a miscarriage (1). Moreover, the incidence of listeriosis has been rising in several European countries (2). Compared with the general population, pregnant women are at markedly increased risk of acquiring listeriosis (3). Women who are infected with *L. monocytogenes* in the third trimester of pregnancy are typically treated with antimicrobial drugs until the child's delivery (3). However, the optimal treatment regimen for listeriosis early in pregnancy is unknown.

We cared for a 28-year-old, previously healthy woman who sought treatment at 12 weeks' gestational age with fever, headache, and neck stiffness; blood cultures were positive for *L. monocytogenes*. Lumbar puncture on admission to our hospital in Boston, Massachusetts, in December 2011, revealed clear fluid and an opening pressure of 15 mm Hg; 1 leukocyte was observed per high-powered field, and cultures of the cerebrospinal fluid were sterile. Pelvic ultrasound showed no abnormalities of the fetus, gestational sac, or uterus.

We treated the patient's condition with intravenous ampicillin